PCT/EP 03 / 10564

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02021696.6

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Anmeldung Nr:
Application no.: 02021696.6
Demande no:

Anmeldetag:
Date of filing: 27.09.02
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
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Polypeptides and nucleic acids encoding these and their use for the prevention, diagnosis or treatment of liver disorders and epithelial cancer

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C12Q1/68

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

ORIDIS BIOMED Forschungs- und Entwicklungs GmbH

September 27, 2002
O37456EP BÖ/HvC

EPO - Munich
58
27. Sep. 2002

Polypeptides and nucleic acids encoding these and their use for the prevention, diagnosis or treatment of liver disorders and epithelial cancer

The invention relates to polypeptides, nucleic acids encoding these or a nucleic acid according to SEQ ID No. 19 and to their use for the diagnosis, prevention and/or treatment of liver disorders and neoplastic disorders especially cancer of the liver and other epithelial tissues, benign liver neoplasms such as adenoma and other proliferative liver disorders such as focal nodular hyperplasia (FNH) and cirrhosis. The invention further relates to methods of diagnosing and treating of these disorders.

The development of cancer in general is characterized by genetic mutations that alter activity of important cellular pathways including, for example, proliferation, apoptosis (cell death), response to stress and epithelial/stroma interactions. It is increasingly recognized that identification of nucleic acids that are deregulated in cancer can provide important new insight into the mechanisms of neoplastic transformation. Identification of deregulated nucleic acid expression in precancerous stages, such as macro regenerative nodules and the "large" and "small" cell change in liver cancer, provide understanding of early events in malignant transformation. Similarly, identification of deregulated gene expression in disorders characterized by tissue proliferation and remodeling, such as FNH and cirrhosis in the liver may distinguish nucleic acids involved in proliferation and malignant transformation. Together such deregulated nucleic acids and the encoded gene products have potential as new diagnostic markers for cancer. Moreover, the products of these deregulated nucleic acids *per se*, are targets for therapeutic intervention in the prevention and/or treatment of these disorders in human patients.

The liver plays a vital role in the metabolism of proteins, lipids, carbohydrates, nucleic acids and vitamins. There are numerous disorders effecting the liver that cannot be diagnosed, prevented or treated effectively, such as hepatocellular carcinoma (HCC). Examination of HCC is particularly well suited for the identification of deregulated gene expression in cancer. This is because tissue samples of HCC can be obtained from surgically resected tumors and the tumors are well circumscribed solid structures with little stromal tissue. Furthermore, as indicated above, there is the possibility for comparative analyses of benign and malignant tumors as well as cirrhosis, a non-neoplastic condition. If the limitations in the art of identifying differentially expressed genes associated with liver disorders could be overcome; this comparative approach may enable identification of deregulated nucleic acids specifically involved in the processes of cellular proliferation and tissue remodeling in a mature organ (e.g., in cirrhosis) as well as the identification and discrimination of gene expression alterations associated with hyperplasia (such as FNH) and with benign and malignant neoplasms (e.g., adenoma and HCC). In HCC there is an urgent need for new and better diagnostic and therapeutic capabilities. Deregulated genes in liver cancer may also be highly relevant to other cancers of the gastrointestinal tract and indeed with other carcinomas (epithelial derived cancers) as these tissues share a common embryological origin.

On a global basis, hepatocellular carcinoma (HCC) belongs to the most common malignant tumors accounting for about 1 million deaths/year. In Southeast Asia the incidence rates are about 150/100,000 population/year with clear male predominance. In western countries 2 to 4 cases/100,000 population are observed annually and the number is still rising. In developing countries, the frequency of HCC correlates with the hepatitis B-virus carrier state, particularly after vertical transmission, affecting younger age groups often in the absence of liver cirrhosis. Elsewhere, HCC is mainly found in older patients with liver cirrhosis most commonly associated with chronic hepatitis C-virus infection and/or chronic alcohol abuse (Ishak et al, 1999.Atlas of Tumor Pathology. Fascicle 31. Armed Forces Institute of Pathology, Washington, DC).

Definitive diagnosis of neoplastic liver disorders such as HCC and many other tumors relies upon histopathological evaluation of biopsy specimens. This invasive surgical procedure is generally not undertaken until symptoms appear and the disease is then most often in advanced stages, thereby limiting therapeutic intervention options. Thus there is a need to improve diagnostics and methods of diagnosis. In addition, early diagnosis is crucial but hampered by late onset or even a lack of specific clinical symptoms. At diagnosis most HCC tumors are no longer amenable to surgical resection (except encapsulated tumors or the fibrolamellar variants) (Chen and Jeng, 1997, J. Gastroenterol. Hepatol. 12: 329-34); moreover, they are highly resistant to cytostatic therapy (Kawata et al., 2001 Br. J. Cancer 84:886-91). Overall, death usually occurs within 1 year after diagnosis. Thus, markers for early detection, prognostic indicators, and effective prevention and/or treatment regimens for HCC are highly desirable in this field.

In contrast, unlike the well-studied situation in colorectal cancer, liver adenoma may not represent a precursor lesion of HCC. Similarly, although cirrhosis and hepatitis viral infections are clearly risk factors for HCC, these conditions are not prerequisite for the development of HCC. Certain liver lesions may represent HCC prestages such as macro regenerative nodular hyperplasia, but this is not yet confirmed (Shortell and Schwartz, 1991, Surg Gynecol Obstet. 173:426-31; Anthony, P. in MacSween et al, eds. Pathology of the Liver. 2001, Churchill Livingstone, Edinburgh). Although these disorders are diagnosed by histopathological investigation of liver resections and liver biopsies, no efficient method exists for earlier or non-invasive detection of these conditions. Again, there is immediate need for diagnostic and prognostic markers for these neoplasms and for non-invasive detection of these disorders.

Within the past decade, several technologies have made it possible to monitor the expression level of a large number of transcripts within a cell at any one time (see, e.g., Schena et al., 1995, Science 270:467-470; Lockhart et al., 1996, Nature Biotechnology 14:1675-1680; Blanchard et al., 1996, Nature Biotechnology 14, 1649; 1996, US 5,569,588). Transcript array technology has been utilized for the identification of genes that are up regulated or down regulated in various disordered states.

Several recent studies have utilized this technology to examine changes in gene expression in human hepatocellular carcinoma. These studies have variously revealed deregulation (i.e., over- and underexpression) of genes encoding liver specific proteins in HCC cell lines and HCC tissues relative to controls. Moreover the studies revealed genes essential for cell cycle control, stress response, apoptosis, lipid metabolism, cell-cell-interaction, DNA repair and cytokine and growth factor production (Graveel et al, 2001, Oncogene 20:2704-12; Kawai et al, 2001, Hepatology 33:676-91; Lau et al, 2000, Oncol. Res. 12:59-69; Nagai et al, 1998, Cancer 82:454-61; Okabe et al, 2001, Cancer Res 61:2129-37; Salvucci et al, 1999, Oncogene 18:181-187; Shirota et al, 2001, Hepatology 33:832-40; Tackels-Horne et al, 2001, Cancer 92: 395-405; Wu et al, 2001, Oncogene 20:2674-3682; Xu et al, 2001, Cancer Res. 61:3176-81). However, there is little concordance in the gene expression patterns reported in these studies that may be due to differences in experimental design and/or to the heterogeneity of HCC tissue *per se*. Moreover, the etiologies of these HCCs are an important factor. Chronic hepatitis B and C virus infections are the major causes of HCC but damage from alcohol and chronic liver metabolic disorders are also recognized to result in HCC and the mechanisms responsible for development of a tumor from these different etiologies are likely to differ. Taken together, until now no satisfactory diagnostics and methods of diagnosing have been developed in order to be able to intervene in liver disorders.

The same applies to the therapy of liver disorders, and epithelial cancers. For HCC for instance, there is no effective therapeutic option except resection and transplantation but these approaches are only applicable in early stages of HCC, limited by the access to donor livers, and associataed with severe risks for the patient. In addition, these approaches are extremely expensive. These cancers respond very poorly to chemotherapeutics, most likely due the normal liver function in detoxification and export of harmful compounds. Several other therapeutic options, such as chemoembolization, cryotherapy and ethanol injection are still in an experimental phase and the efficacy of these is not established. Surgical intervention remains the best treatment option but it is not possible to define with precision the extent of the tumor. This invasive procedure therefore, is suboptimal from the perspective of treatment. Furthermore, the lack of early diagnostics for specific liver dysfunctions

leads most often to advanced progression of the disease that further confounds therapeutic options and dramatically increases patient mortality from these diseases (Jansen P.L., 1999, Neth. J. Med. 55:287-292). Thus until now no satisfactory therapies have been developed in order to be able to intervene in liver disorders, and other epithelial cancers. Furthermore, in the state of the art, a recognition of the different subtypes of liver disorders such as HCC precursor lesions, benign liver neoplasms, and metabolic liver diseases such as alcoholic liver disease and cirrhosis, as revealed by differential gene expression, have not been disclosed.

A summary of the key disease features of some of the disorders evaluated in the invention is provided in the following table.

DISORDER	Cellular proliferation	Tissue remodeling	Clonal cell expansion	Neoplasia	Transformation/ Malignant potential
Cirrhosis	+	+			
FNH	+	+	+/-		
Adenoma	+	+	+	+	
HCC	+	+	+	+	+

It is, therefore, an object of the present invention to make available diagnostics, methods of diagnosis, pharmaceutical compositions and methods of prevention and/or treatment of liver disorders, especially of hepatocellular carcinoma (HCC), and epithelial cancers, pre-cancerous liver lesions, benign neoplasms such as adenoma, hyperplasias (i.e., the focal nodular variety) and cirrhosis that overcome the limitations present in the art. Suprisingly the evaluation of multiple liver disorders with overlapping but distinct morphological and clinical features provided new information for identification and discrimination and ultimately therapeutic strategies for disorders of the liver.

The object of the invention is solved by a method of diagnosis of a liver disorder, liver cancer and/or epithelial cancer, wherein at least one compound selected from the group consisting of a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, an antibody directed against the polypeptide, and a fragment of the antibody, is identified in the sample of a patient and compared with at least one compound of a reference library or of a reference sample.

The object of the invention is also solved by a method of treating a patient suffering from a liver disorder or an epithelial cancer, wherein at least one component selected from the group consisting of a polypeptide according SEQ ID No. 1-9, a functional variant of the polypeptide, a nucleic acid encoding the polypeptide, a nucleic acid encoding the functional variant, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against one of the aforementioned polypeptides, an antibody directed against a functional variant of one of the aforementioned polypeptides, a fragment of one of the aforementioned antibodies, a vector containing a nucleic acid coding for one of the aforementioned antibodies, a vector containing a nucleic acid coding for one of the aforementioned antibody fragments, a cell containing the vector containing a nucleic acid coding for one of the aforementioned antibodies, and a cell containing the vector containing a nucleic acid coding for one of the aforementioned antibody fragments, is administered to the patient in need of a the treatment in a therapeutically effective amount.

In another aspect of the invention it is provided a pharmaceutical composition containing at least one compound selected from the group consisting of a polypep-

tide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide or a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of

A subset of these nucleic acids and polypeptides according to the invention have further been shown to be specifically expressed or deregulated in other cancers of epithelial origin and preferably not in corresponding normal human tissue(s). These nucleic acids preferably include SEQ ID Nos. 11-16, and 19 (Figure 25). Deregulated nucleic acids in liver cancer, in particular may preferably be highly relevant to other cancers of the gastrointestinal tract as these tissues share a common embryological origin. Consequently, these nucleic acids and the encoded polypeptides may preferably be similarly utilized for diagnostics methods of diagnosis, pharmaceutical compositions and methods of prevention and/or treatment of these epithelial cancers.

The polypeptides and nucleic acids according to the invention have in common that they are differentially expressed in a sample isolated from a patient suffering from a disorder according to the invention compared to a reference sample. Preferably the regulation of the polypeptides and nucleic acids according to the invention is essential for the pathologic process and which are thus in a direct or indirect relationship with diagnosis, prevention and/or treatment of disorders according to the invention. The polypeptides and the nucleic acids according to the invention preferably do not belong to the targets known until now such that suprising and completely novel approaches for diagnosis and therapy result from this invention.

Generally, the analysis of differentially expressed genes in tissues is less likely to result in errors in the form of artifactual false-positive clones than the analysis of cell culture systems. In addition to the fact that existing cell culture systems cannot adequately simulate the complexity of pathological processes in the tissue, the variations in cell behavior in the culture environment lead to nucleic acid and polypeptide expression patterns with questionable relation to the actual pathologic state.

These problems may be less pronounced by an approach that utilizes gene expression in normal and diseased human tissue but again multiple variables confound clear identification of differential gene expression that is directly relevant to disease. For example, differentially expressed nucleic acids may result from inter-individual differences, metabolic state and/or clinical treatment paradigm. Further, large scale gene expression studies using cDNA microarrays do not indicate the cellular source of variation in gene expression. In addition, a differential gene expression study including all or most genes produces a very large volume of data that confounds identification of key disease-associated gene expression changes. Consequently, an approach that includes large scale profiling of gene expression from tissue from liver disorders that are defined only generally (as for example, 'liver tumors') is unlikely to illuminate key genes involved in the disease process and it is these key genes that represent best targets for diagnostics and therapeutic intervention.

On account of these difficulties, the success of the screening was significantly dependent on the choice of the experimental parameters. While the methods used are based on established procedures, the screening and verification strategy is already inventive *per se* owing to the elaborate and defined choice of parameters. A unique approach employed in this invention has utilized discrete, pathologist-confirmed liver cancer pathologies for production of disease specific cDNA libraries enriched in nucleic acids specifically up- and down-regulated in HCC compared with a pool of non-neoplastic human livers. Non-diseased reference liver samples for the experiments was also diagnostically confirmed and pooled from 3 independent samples to reduce detection of false positives resulting from inter-individual variations. Nucleic acids commonly expressed at similar levels in the reference liver pool and in diseased liver (i.e., HCC) were removed by the generation of subtractive suppressive hybridization (SSH) cDNA libraries (Diatchenko et al., 1996, Proc. Natl. Acad. Sci. USA 93:6025-6030). These cDNAs are highly enriched for nucleic acids both up- and down-regulated in HCC but do not represent those that are not differentially expressed. Each of several thousand SSH clones were amplified by the polymerase chain reaction (PCR) and affixed to glass slides in custom cDNA microarrays. RNA from additional pathologist-confirmed liver disorders was con-

verted to fluorescently-labeled cDNA for competitive hybridization with the pooled non-diseased liver RNA on the microarrays. The resulting ratio of hybridization intensity revealed nucleic acids specifically deregulated in liver disorders. In addition to providing a pool of candidate cDNAs highly enriched for differentially expressed genes, the SSH library represents on a genome-wide scale most if not all differentially expressed genes with far fewer clones than in standard cDNA libraries. This feature thereby focuses on nucleic acids specifically deregulated in disease. Furthermore, the SSH libraries generated in this invention include cDNA clones from nucleic acids that are essentially not expressed in normal liver and thereby not represented in conventional cDNA libraries or on genome-scale cDNA microarrays.

In a first aspect the invention relates to a polypeptide comprising a sequence according to the SEQ ID No. 2 or a functional variant thereof. The invention also relates to a nucleic coding for the polypeptide or a functional variant thereof, in particular to the nucleic acid according to the SEQ ID No. 11 and variants thereof.

The nucleic acids and polypeptides according to the invention can be utilized for the diagnosis, prevention and treatment of liver disorders, and epithelial cancers.

In a preferred embodiment the polypeptide consists of the sequence according to the SEQ ID No. 2. In another preferred embodiment the nucleic acid consists of the SEQ ID No. 11.

Compared to the state of the art, these polypeptides and nucleic acids surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis of the liver disorders and/or epithelial cancers.

In another aspect of the invention the invention relates to the use of at least one polypeptide according SEQ ID No. 1-9, at least one functional variant of the polypeptide, at least one nucleic acid encoding the polypeptide, at least one nucleic acid

encoding the functional variant, at least one nucleic acid coding for the SEQ ID No. 19, at least one variant of one of the aforementioned nucleic acids, at least one nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, at least one nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, at least one vector containing one of the aforementioned nucleic acids, at least one cell containing one of the aforementioned nucleic acids, at least one cell containing the vector, at least one antibody directed against one of the aforementioned polypeptides, at least one antibody directed against a functional variant of one of the aforementioned polypeptides, at least one fragment of one of the aforementioned antibodies, at least one vector containing a nucleic acid coding for one of the aforementioned antibodies, at least one vector containing a nucleic acid coding for one of the aforementioned antibody fragments, at least one cell containing the vector containing a nucleic acid coding for one of the aforementioned antibodies, and/or at least one cell containing the vector containing a nucleic acid coding for one of the aforementioned antibody fragments, for the diagnosis, prevention and/or treatment of disorders according to the invention. Further embodiments of the invention are described in detail below.

When compared to the state of the art of therapy of liver disorders, and/or epithelial cancers the use of these components surprisingly provide an improved, sustained and/or more effective diagnosis, prevention and/or treatment of disorders according to the invention.

The term "polypeptide" refers to the full length of the polypeptide according to the invention. In a preferred embodiment the term "polypeptide" also includes isolated polypeptides and polypeptides that are prepared by recombinant methods, e.g. by isolation and purification from a sample, by screening a library and by protein synthesis by conventional methods, all of these methods being generally known to the person skilled in the art. Preferably, the entire polypeptide or parts thereof can be synthesized, for example, with the aid of the conventional synthesis such as the Merrifield technique. In another preferred embodiment, parts of the polypeptides according to the invention can be utilized to obtain antisera or specific monoclonal antibodies, which may be used to screen suitable gene libraries prepared to express

the encoded protein sequences in order to identify further functional variants of the polypeptides according to the invention.

The term "polypeptide according to the invention" refers to the polypeptides according to the SEQ ID No. 1 to SEQ ID No. 9.

The term "functional variants" of a polypeptide within the meaning of the present invention refers to polypeptides which have a sequence homology, in particular a sequence identity, of about 70%, preferably about 80%, in particular about 90%, especially about 95%, most preferred of 98 % with the polypeptide having the amino acid sequence according to one of SEQ ID No. 1 to SEQ ID No. 9. Such functional variants are, for example, the polypeptides homologous to a polypeptide according to the invention, which originate from organisms other than human, preferably from non-human mammals such as, for example mouse, rats, monkeys and pigs. Other examples of functional variants are polypeptides which are encoded by different alleles of the gene, in different individuals, in different organs of an organism or in different developmental phases. Functional variants, for example, also include polypeptides which are encoded by a nucleic acid which is isolated from non-liver-tissue, e.g. embryonic tissue, but after expression in a cell involved in liver disorders have the designated functions. Functional variants preferably also include naturally occurring or synthetic mutations, particularly mutations that quantitatively alter the activity of the peptides encoded by these sequences. Further, such variants may preferably arise from differential splicing of the encoding gene.

"Functional variants" refer to polypeptides that have essentially the same biological funtion(s) as the corresponding polypeptide according to the invention. Such biological function can be assayed in a functional assay.

In order to test whether a candidate polypeptide is a functional variant of a polypeptide according the invention, the candidate polypeptide can be analyzed in a functional assay generally known to the person skilled in the art, which assay is

suitable to assay the biological function of the corresponding polypeptide according to the invention. Such functional assay comprise for example cell culture systems; the generation of mice in which the genes are deleted ("knocked out") or mice that are transgenic for gene encoding the candidate polypeptide; enzymatic assays, etc. If the candidate polypeptide demonstrates or directly interferes with essentially the same biological function as the corresponding polypeptide according to the invention, the candidate polypeptide is a functional variant of the corresponding polypeptide, provided that the candidate polypeptide fulfills the requirements on the level of % sequence identity mentioned above.

Furthermore, the term "functional variant" encompasses polypeptides that are preferably differentially expressed in patients suffering from liver disorders, or other epithelial cancers relative to a reference sample or a reference library, including polypeptides expressed from mutated genes or from genes differentially spliced, provided that the candidate functional variant polypeptide fulfills the criteria of a functional variant on the level of % sequence identity. Such expression analysis can be carried out by methods generally known to the person skilled in the art.

"Functional variants" of the polypeptide can also be parts of the polypeptide according to the invention with a length of at least from about 7 to about 1000 amino acids, preferably the polypeptide is comprised of at least 10 amino acids, more preferably at least 20, most preferred at least 50, for example at least 100, for example at least 200, for example at least 300, for example at least 400, for example at least 500, for example at least 600 amino acids provided that they have essentially the same biological function(s) as the corresponding polypeptide according to the invention. Also included are deletions of the polypeptides according to the invention, in the range from about 1-30, preferably from about 1-15, in particular from about 1-5 amino acids provided that they have essentially the same biological function(s) as the corresponding polypeptide according to the invention. For example, the first amino acid methionine can be absent without the function of the polypeptide being significantly altered. Also, post-translational modifications, for example lipid anchors or phosphoryl groups may be present or absent in variants.

"Sequence identity" refers to the degree of identity (% identity) of two sequences, that in the case of polypeptides can be determined by means of for example BLASTP 2.0.1 and in the case of nucleic acids by means of for example BLASTN 2.014, wherein the Filter is set off and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402).

"Sequence homology" refers to the similarity (% positives) of two polypeptide sequences determined by means of for example BLASTP 2.0.1 wherein the Filter is set off and BLOSUM is 62 (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402).

The term "liver disorder" refers to and comprises all kinds of disorders that preferably affect the anatomy, physiology, metabolic, and/or genetic activities of the liver, that preferably affect the generation of new liver cells, and/or the regeneration of the liver, as a whole or parts thereof preferably transiently, temporarily, chronically or permanently in a pathological way. Preferably also included are inherited liver disorders and neoplastic liver disorders. Liver disorder is further understood to preferably comprise liver disorders caused by trauma, intoxication, in particular by alcohol, drugs or food intoxication, radiation, infection, cholestasis, immune reactions, and by inherited metabolic liver diseases. Preferred examples of liver liver disorders include cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, and heamochromatosis. Preferably further included are autoimmune-disorders wherein the autoimmune response is directed against at least one polypeptide according to the invention. Within the meaning of the present invention the term "liver disorder" preferably also encompasses liver cancer, for example hepatocellular carcinoma (HCC), benign liver neoplasms such as adenoma and/or FNH. Preferably HCC further comprises subtypes of the mentioned disorders, preferably including liver cancers characterized by intracellular proteinaceous inclusion bodies, HCCs characterized by hepatocyte steatosis, and fibrolamellar HCC. For example, precancerous lesions are preferably also included such as those characterized by increased hepatocyte cell size (the "large cell" change), and those characterized by decreased hepatocyte cell size (the "small cell" change) as well as macro

regenerative (hyperplastic) nodules (Anthony, P. in MacSween et al, eds. Pathology of the Liver. 2001, Churchill Livingstone, Edinburgh).

The term "epithelial cancer" within the meaning of the invention includes adenocarcinomas of any organ other than the liver preferably of the lung, stomach, kidney, colon, prostate, skin and breast, and refers to disorders of these organs in which epithelial cell components of the tissue are transformed resulting in a malignant tumor identified according to the standard diagnostic procedures as generally known to a person skilled in the art.

Within the meaning of the invention the term "disorder according to the invention" encompasses epithelial cancer and liver disorders as defined above.

In the case of polypeptides, the term "differential expression of a polypeptide" refers to the relative level of expression of the polypeptide in an isolated sample from a patient compared to the expression of the polypeptide in a reference sample or a reference library. The expression can be determined by methods generally known to the person skilled in the art. Examples of such methods include immunohistochemical or immunoblot or ELISA detection of the polypeptide with antibodies specific for the polypeptide. Detection of the polypeptide through genetic manipulation to label the polypeptide and detection in a model system is preferably also included such as by tagging the polypeptide in a transgene for expression in a model system.

The term "sample" refers to a biomaterial comprising liver tissue or liver cells, preferably tissue from another organ subject to malignant transformation or a cell from this organ, blood, serum, plasma, ascitic fluid, pleural effusions, cerebral spinal fluid, saliva, urine, semen, feces.

The sample can be isolated from a patient or another subject by means of methods including invasive or non-invasive methods. Invasive methods are generally known

to the skilled artisan and comprise for example isolation of the sample by means of puncturing, surgical removal of the sample from the opened body or by means of endoscopic instruments. Minimally invasive and non-invasive methods are also known to the person skilled in the art and include for example, collecting body fluids such as blood, serum, plasma, ascitic, pleural and cerebral spinal fluid, saliva, urine, semen, and feces. Preferably the non-invasive methods do not require to penetrate or open the body of a patient or subject through openings other than the body openings naturally present such as the mouth, ear, nose, rectum, urethra, and open wounds.

The term "minimally invasive" procedure refers to methods generally known, especially by persons skilled in the art, for obtaining patient sample material that do preferably not require anesthesia, can be routinely accomplished in a physician office or clinic and are either not painful or only nominally painful. The most common example of a minimally invasive procedure is venupuncture.

The term "reference sample" refers to a sample that serves as an appropriate control to evaluate the differential expression of a nucleic acid and/or a polypeptide according to the invention in a given sample isolated from a patient; the choice of such appropriate reference sample is generally known to the person skilled in the art. Examples of reference samples include samples isolated from a non-diseased organ or tissue or cell(s) of the same patient or from another subject, wherein the non-diseased organ or tissue or cell(s) is selected from the group consisting of liver tissue or liver cells, blood, or the samples described above. For comparison to expression in the sample isolated from a patient with the liver disorder, the reference sample may also include a sample isolated from a non-diseased organ or tissue or cell(s) of a different patient, wherein the liver disordered- tissue or cell(s) is selected from the sample group listed above. Moreover the reference may include samples from healthy donors, preferably matched to the age and sex of the patient.

The term "reference library" refers to a library of clones representing expressed genes, which, library is preferably prepared from non-diseased liver tissue or cells.

The reference may also derive from mRNA from non-diseased liver tissue or cells and may also comprise a data base containing data on non-diseased tissue expression of nucleic acids. For comparison of the expression of the nucleic acids or polypeptides according to the invention, a sample isolated from a patient with the disordered liver, the reference library may comprise an expression library prepared from liver disorder-diseased liver tissue or cells and a data base containing data on liver disorder-specific expression of nucleic acids.

The term "patient" within the meaning of the invention includes animals, preferably mammals, and humans, dead or alive. The patient is either suffering from a liver disorder, and/or an other epithelial cancer, subject to analysis, preventive measures, therapy and/or diagnosis in the context of liver disorder and/or an other epithelial cancer.

The term "subject" within the meaning of the invention includes animals, preferably mammals, and humans, dead or alive that are not suffering from a liver disorders and/or an other epithelial cancer and thus represent a preferred appropriate control for the determination of differential expression of nucleic acids and/or polypeptides according to the invention in a patient.

The term "effective treatment" within the meaning of the invention refers to a treatment that preferably cures the patient from at least one disorder according to the invention and/or that improves the pathological condition of the patient with respect to at least one symptom associated with the disorder, preferably 3 symptoms, more preferably 5 symptoms, most preferably 10 symptoms associated with the disorder; preferably on a transient, short-term (in the order of hours to days), long-term (in the order of weeks, months or years) or permanent basis, wherein the improvement of the pathological condition may be preferably constant, increasing, decreasing, continuously changing or oscillatory in magnitude as long as the overall effect is a significant improvement of the symptoms compared with a control patient. Therapeutic efficacy and toxicity, e.g. ED_{50} and LD_{50} may be determined by standard pharmacological procedures in cell cultures or experimental animals.

The dose ratio between therapeutic and toxic effects is the therapeutic index and may be expressed by the ratio LD_{50}/ED_{50}. Pharmaceutical compositions which exhibit large therapeutic indexes are preferred. the dose must be adjusted to the age, weight and condition of the individual patient to be treated, as well as the route of administration, dosage form and regimen, and the result desired, and the exact dosage should of course be determined by the practitioner.

The actual dosage depend on the nature and severity of the disorder being treated, and is within the discretion of the physician, and may be varied by titration of the dosage to the particular circumstances of this invention to produce the desired therapeutic effect. However, it is presently contemplated, that pharmaceutical compositions containing of from about 0.1 to 500 mg of the active ingredient per individual dose, preferably of from about 1 to 100 mg, most preferred from about 1 to 10 mg, are suitable for therapeutic treatments.

The active ingredient may be administered in one or several dosages per day. A satisfactory result can, in certain instances, be obtained at a dosage as low as 0.1 µg/kg intravenously (i.v.) and 1 µg peroral (p.o.). Preferred ranges are from 0.1 µg/kg/day to about 10 mg/kg/day i.v., and from 1 µg/kg/day to about 100 mg/kg/day p.o.

In another aspect the invention relates to a fusion protein containing a polypeptide according to the SEQ ID No. 1 to 9 or a functional variant thereof.

A "fusion protein" refers to a polypeptide containing at least one polypeptide according to the SEQ ID No. 1 to 9, a functional variant or part thereof and at least one component A selected from polypeptide, peptide and/or peptide analogue that is linked to the polypeptide according to the invention by means of covalent or non-covalent binding such as e.g. hydrogen bonds, generally known to the person skilled in the art. Preferred examples of component A for fusion proteins are polypeptide, peptide and/or peptide analogues, that facilitate easier detection of the fusion proteins, these are, for example, "green-fluorescent-protein" or variants the-

reof. Also included are fusion proteins that facilitate purification of the recombinant protein such as "his-tags", or fusions that increase the immunogenicity of the protein.

Fusion proteins according to the invention can be produced by methods generally known to the person skilled in the art. The fusion proteins according to the invention can be used for the diagnosis, prevention and or treatment of liver disorders and/or epithelial cancer.

Compared to the state of the art, these fusion proteins surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or epithelial cancers.

In a further embodiment of the invention a nucleic acid encoding a polypeptide comprising a polypeptide according to the invention or a functional variant thereof or a nucleic acid according to SEQ ID No. 19, or a variant thereof is provided. Preferred nucleic acids according to the invention have a sequence according to one of SEQ ID No. 10 to SEC ID No. 18 or a nucleic acid according to SEQ ID No. 19, or a variant thereof. In particular the invention relates to nucleic acids according to the invention that have been isolated.

Compared to the state of the art, these nucleic acids and polypeptides surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or epithelial cancers.

The term "nucleic acid according to the invention" refers to the nucleic acids according to the SEQ ID No. 10 to SEQ ID No. 19.

The term "encoding nucleic acid" relates to a DNA sequence which codes for an isolatable bioactive polypeptide according to the invention or a precursor thereof. The polypeptide can be encoded by a sequence of full length or any part of the coding sequence as long as the biological function, such as for example receptor-activity, is essentially retained (cf. definition of functional variant).

It is known that small alterations in the sequence of the nucleic acids described above can be present, for example, due to the degeneration of the genetic code, or that untranslated sequences can be attached to the 5' and/or 3' end of the nucleic acid without significantly affecting the activity of the encoded polypeptide. This invention, therefore, also comprises so-called naturally occurring and artificially generated "variants" of the nucleic acids described above.

Preferably, the nucleic acids used according to the invention are DNA or RNA, preferably a DNA, in particular a double-stranded DNA. In particular the nucleic acid according to the invention may be an RNA molecule, preferably single-stranded or a double-stranded RNA molecule. The sequence of the nucleic acids may further comprise at least one intron and/or one polyA sequence.

Nucleic acids according to the invention can be produced by methods generally known to the skilled artisan and have also been described in detail below.

"Variant" within the meaning of the invention refers to all DNA sequences which are complementary to a DNA sequence, which hybridize with the reference sequence under stringent conditions and have a similar activity to the corresponding polypeptide according to the invention. The nucleic acids according to the invention can also be used in the form of their antisense sequence.

"Variant" of the nucleic acids can also be parts of the nucleic acid according to the present invention with at least about 8 nucleotides length, preferably with at least about 16 nucleotides length, in particular with at least about 21 nucleotides length,

more preferably with at least about 30 nucleotides length, even more preferably with at least about 40 nucleotides length, most preferably with at least about 50 nucleotides length as long as the parts have a similar activity to the corresponding polypeptide according to the invention. Such activity can be assayed using the functional assays described further above.

In a preferred embodiment of the invention the nucleic acid comprises a nucleic acid having a sequence complementary to a nucleic acid according to the invention or a variant thereof. Preferably the nucleic acid comprises a non-functional mutant variant of the nucleic acid according to the invention or a variant thereof.

In particular the invention relates to a nucleic acid having a complementary sequence wherein the nucleic acid is an antisense molecule or an RNA interference molecule.

The term "non-functional mutant variant of a nucleic acid" refers to a nucleic acid derived from a nucleic acid according to the invention or a variant thereof having been mutated such that the polypeptide encoded by the non-functional mutant variant of the nucleic acid exhibits a biological activity which in comparison the non-mutated polypeptide is significantly decreased or abolished. Such activity of the polypeptide encoded by the non-functional mutant variant nucleic acid can be determined by means of a functional assay as described above for the evaluation of functional variants. The construction and screening of such non-functional mutant variant derived from a nucleic acid according to the invention are generally known to the person skilled in the art. Such "non-functional mutant variant of a nucleic acid" according to the invention can be expressed in a patient and will preferably abolish or diminish the level of expression of the targeted nucleic acid by competing with the native mRNA molecules for translation into polypeptides by the ribosomes.

"Stringent hybridization conditions" refer to those conditions in which hybridization takes place at 60°C in 2.5 × SSC buffer and remains stable following a number of washing steps at 37°C in a buffer of lower salt concentration.

The term "differential expression of a nucleic acid" refers to the relative level of expression of the nucleic acid in an isolated sample from a patient compared to the expression of the nucleic acid in a reference sample or a reference library. Definitions of reference samples and reference libraries have been described in detail above. The expression can be determined by methods generally known to the person skilled in the art. Examples of such methods include RNA blot (northern) analysis, nuclease protection, in situ hybridization, reverse transcriptase PCR (RT-PCR; including quantitative kinetic RT-PCR). cDNA and oligonucleotide microarrays are also included as such methods.

In a preferred embodiment the nucleic according to the invention is the OBcl1 cDNA (SEQ ID 10), which was assembled by identification of overlapping sequences from the non-redundant and human EST GenBank sequence databases. The expression in HCC of RNA corresponding to each assembled sequence was confirmed experimentally. The initial sequence upregulated in HCC relative to non-diseased liver identified as an SSH cDNA clone corresponded to GenBank sequence AL050205. The 5' end of that sequence overlaps with AF131755; this sequence was extended progressively 5' with XM113703, AK055521 and AY004310. The latter three sequences include the open reading frame encoding OBcl1.pr (SEQ ID 1). In support of this mRNA construct, all overlapping cDNA sequences can be localized to the same chromosome. Furthermore, an mRNA of approximately 6 kilobases was identified by RNA blot analysis of HCC but not normal liver RNA using the SSH sequence from this clone as a hybridization probe (Figure 27). Expression of sequences corresponding to this clone have not previously been reported in liver or in HCC.

In a preferred embodiment the polypeptide according to the invention is the OBcl1.pr polypeptide sequence (SEQ ID 1) which was surprisingly identified from

an mRNA identified to be upregulated in HCC by an average of 2.9-fold relative to non-diseased liver (OBcl1, SEQ ID 10). cDNA sequences encoding this polypeptide and overlapping with this mRNA were identified with reverse transcriptase PCR analysis and these nucleic acids are similarly elevated in HCC. This polypeptide sequence was previously unrecognized with respect to elevated levels in HCC. From the sequence of the OBcl1.pr polypeptide, two conserved sequence domains can be identified with the conserved domain prediction CDD algorithm available with the BLAST sequence analysis tools (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402); a lupus La polypeptide type RNA binding domain (SEQ ID No. 1, amino acids 47 to 125), and a GTPase enzymatic domain with unknown function (SEQ ID No. 1, amino acids 90 to 203). The OBcl1.pr sequence has been designated in the GenBank sequence database as the cellular myeloproliferative leukemia receptor (c-Mpl) binding polypeptide. Although a potential modulator of the myeloproliferative leukemia virus receptor (also known as the thrombopoietin receptor), the functional role for this polypeptide has not been described in any system. Similarly, the expression pattern of this polypeptide has not been disclosed. The mRNA encoding this polypeptide is elevated more than 2-fold relative to non-diseased liver in 11 of 21 liver tumors subjected to expression profiling (52%). The mRNA encoding this polypeptide is similarly elevated at least 2-fold in 4 of 4 focal nodular hyperplasia (FNHs) profiled (100%) (Figure 21). For this and the other nucleic acids according to the invention, this value for expression includes the expression value ratio data from all of the 21 HCC samples subjected to the cDNA microarray expression profiling experiments, including the values from samples that were not elevated by 2-fold or greater.

The expression of this mRNA is remarkably specific to liver disorders since expression is not detected in other carcinomas analyzed nor in non-diseased tissues including liver, kidney, stomach, lung, skin and others (see Figure 25). Therefore it was surprisingly found that there is a strong and specific correlation between the expression of OBcl1.pr polypeptide (SEQ ID No.1) and the nucleic acid encoding the polypeptide (SEQ ID No. 10) respectively and the disorders according to the invention. Therefore the polypeptide and the encoding nucleic acid can be utilized for diagnosis of disorders according to the invention, for example for the diagnostic

discrimination between hyperplastic (including neoplastic) liver diseases and cirrhosis. Furthermore these results demonstrate that OBcl1.pr polypeptide (SEQ ID No.1) and the nucleic acid encoding the polypeptide (SEQ ID No. 10) can be employed in the prevention and therapy of disorders according to the invention, in particular for the treatment of hyperplastic (including neoplastic) liver diseases. With regard to the treatment it is preferred to carry out the treatment such that the expression of the OBcl1.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the OBcl1.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the OBcl1.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the OBcl1.pr polypeptide or an antibody fragment thereof which block the activity of the OBcl1.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this OBcl1.pr polypeptide and/or OBcl1 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or epithelial cancers.

In a preferred embodiment the nucleic acid according to the invention is the OBcl5 (SEQ ID 11) which is the compiled sequence encoding OBcl5pr. (SEQ ID 2). The discrete nucleic acid sequence as well as the deduced polypeptide sequence are novel. The entire sequence was established from a number of GenBank expressed sequence tag (EST) database sequences and GenBank genomic database sequences and each segment was verified for overexpression in HCC. For example, the sequence for this nucleic acid on a cDNA microarray is elevated an average of 24.7-fold relative to non-diseased liver reference (Figure 21). Expression of partial sequences corresponding to this clone has been reported in several tissues and some tumors (including fetal liver and colon adenocarcinoma) but the entire sequence has not previously been implicated in HCC.

Information concerning expression of this and all sequences according to the invention is obtained from searching of public domain databases (such as the Pub-

Med and SOURCE). Journal articles have not been published for most of the sequences according to the invention. The relative abundance of cDNA clones from automatically sequenced cDNA libraries therefore provides the evidence cited herein for expression of this and other sequences according to the invention. This information is accessed via databases such as 'SOURCE' (provided by the Genetics Department, Stanford University) that includes data curated from UniGene, SwissProt, GeneMap99, RHdb, dbEST, GeneCards and LocusLink databases.

In a preferred embodiment the polypeptide according to the invention is the OBcl5.pr polypeptide (SEQ ID 2) which represents the largest open reading frame from this deregulated mRNA sequence. This polypeptide sequence does not contain recognized sequence homologies to characterized polypeptides or to known structural motifs. No pattern of expression has been described for this polypeptide. Expression of the mRNA encoding this polypeptide is elevated greater than 2 fold in 100% of HCC cases examined relative to non-diseased liver and greater than 8-fold in 17 of the 21 cases profiled (81%). Elevated expression of the encoding mRNA relative to non-diseased liver is also evident in liver adenoma, FNH, and cirrhotic livers but the transcript is less dramatically upregulated in cirrhosis. The mRNA encoding this polypeptide is expressed in non-diseased human lung, brain (cortex), colon, testis tissue but not in most other carcinomas evaluated. Overexpression of this polypeptide and/or the encoding mRNA, therefore may be useful for diagnosis of liver disorders. These results clearly demonstrate that the OBcl5.pr polypeptide (SEQ ID 2) and the nucleic acid encoding the polypeptide (SEQ ID 11) can be utilized for diagnosis, prevention and treatment of disorders according to the invention, in particular for HCC, liver adenoma, FNH and cirrhosis. With regard to the treatment it is preferred to carry out the treatment such that the expression of the OBcl5.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the OBcl5.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the OBcl5.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the OBcl5.pr polypeptide or an antibody fragment thereof which block the activity of the OBcl5.pr polypeptide to a patient in need of such treatment. Compared to the state

of the art, this OBcl5.pr polypeptide and/or OBcl5 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In a preferred embodiment the nucleic acid according to the invention is the IK2 (SEQ ID 12) is represented by the Gene Bank sequence NM_025160 which includes the open reading frame encoding IK2.pr polypeptide (SEQ ID 3). The IK2.pr polypeptide is another embodiment of the invention. EST sequences corresponding to this clone have been reported in cDNA libraries from several tissues including liver and in adenocarcinomas, but the sequence has not previously been implicated in HCC. Expression of this polypeptide has not been described in any cell or tissue. The polypeptide sequence has no known function although the sequence is evolutionarily well conserved (predicted polypeptides are found in several mammals, fruit fly (*Drosophila*) and plants (*Arabidopsis*). The CDD algorithm predicts several WD40-type polypeptide-polypeptide interaction domains in this polypeptide sequence according to the invention. In liver samples from HCC patients expression of the mRNA encoding this polypeptide is surprisingly elevated relative to non-diseased liver by an average value of 4.67-fold in 15 of the 21 cases profiled (71%). Elevated expression of the encoding mRNA relative to non-diseased liver is also evident in cirrhotic livers. Highest differential expression levels of the mRNA encoding this peptide relative to non-diseased liver were observed in FNH; 8-fold upregulation in 4 of 4 cases profiled. The mRNA encoding this polypeptide is also expressed in several other human carcinomas including those of the mammary gland, lung and kidney, and in 2 (breast and kidney) of the 17 non-diseased human tissues examined. These results demonstrate that the overexpression of this polypeptide and/or the encoding mRNA, can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC, FNH, cirrhosis, and epithelia-derived neoplasms. With regard to the treatment it is preferred to carry out the treatment such that the expression of the IK2.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid

encoding the IK2.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the IK2.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the IK2.pr polypeptide or an antibody fragment thereof which block the activity of the IK2.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this IK2.pr polypeptides and/or IK2 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In a preferred embodiment the nucleic acid according to the invention is the IK5 cDNA sequence (SEQ ID 13) which represents the sequence of an HCC deregulated cDNA clone. Expression of sequences corresponding to this clone has been reported in several tissues (including liver) and some tumors (including pituitary and prostate) but the sequence has not previously been described to be upregulated in HCC. In a preferred embodiment the polypeptide according to the invention is the IK5.pr polypeptide (SEQ ID 4) which is encoded by the IK5 cDNA sequence (SEQ ID 13). The polypeptide sequence is deduced from the GenBank database (Accession number: NM_006407) as JWA, a vitamin A responsive polypeptide. Although the gene encoding this putative polypeptide has been described from stimulation of cultured cells with vitamin A, the presence of the polypeptide has not been described in any cell or tissue and the function is unknown. JWA is further described as a cytoskeleton-associated polypeptide in the GenBank database. The polypeptide shares homology also with rodent polypeptides that interact specifically with and may reduce the activity of the EAAC1 glutamate transporter. A conserved domain search of this sequence indicates the likely presence of a prenylated rab acceptor 1 domain (PRA1), possibly mediating interaction with G protein signaling molecules. Expression of the mRNA encoding this polypeptide is elevated by an average of 9.14-fold relative to non-diseased liver in 100% of the HCC cases profiled. Similarly, elevated expression of the encoding mRNA is also evident in Adenoma and FNH. The encoding mRNA expression is differentially expressed also in cirrhotic livers but to a lesser extent than in the other liver disorders. The mRNA encoding this polypeptide is expressed in lung, kidney and colon human carcinomas but in just 1 of the 17 non-diseased human tissues examined.

Overexpression of this polypeptide and/or the encoding mRNA, may mark specific epithelia-derived neoplasms, including liver cancer. These results show that the differential upregulated expression of the IK5 cDNA sequence is highly specific for disorders according to the invention. Therefore the IK5.pr polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC, Adenoma, FNH, cirrhosis, and epithelia-derived neoplasms. With regard to the treatment it is preferred to carry out the treatment such that the expression of the IK5.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the IK5.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the IK5.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the IK5.pr polypeptide or an antibody fragment thereof which block the activity of the IK5.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this IK5.pr polypeptide and/or IK5 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In a preferred embodiment the nucleic acid according to the invention is the SEQ ID 14 which has been disclosed before (Accession. no. X83544) encoding the DAP3.pr polypeptide (SEQ ID 5). The invention further relates to the death associated polypeptide 3 (DAP3, SEQ ID 5) which has been implicated in promotion of apoptotic cell death when overexpressed in cultured cells (Kissil et al., 1995, J. Biol. Chem., 270:27932-6). This polypeptide has not been implicated, however, in disorders according to the invention, especially in liver disorders or HCC. The polypeptide contributes to the mitochondrial 28S ribosomal complex. As such, this polypeptide is likely to be ubiquitously expressed in many if not all tissues and cells, albeit apparently at relatively low levels. No specific function for endogenous DAP3 has not been described (Cadvar Koc et al., 2001, FEBS Lett., 492:166-170). Expression of the mRNA encoding this polypeptide is elevated an average of 5.5-fold relative to non-diseased liver in 18 of the 21 HCC cases profiled (86%).

Elevated expression of the encoding mRNA is also evident in other liver disorders but to a lesser extent than in HCC. These results show that the strongly upregulated expression of the DAP3 cDNA sequence is highly specific for disorders according to the invention, especially in HCC. Therefore the DAP3 polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC. With regard to the treatment it is preferred to carry out the treatment such that the expression of the DAP3 polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the DAP3 polypeptide. Alternatively the treatment may be carried out such that the activity of the DAP3 polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the DAP3 polypeptide or an antibody fragment thereof which block the activity of the DAP3 polypeptide to a patient in need of such treatment. Compared to the state of the art, this DAP3 polypeptide and DAP3 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In another preferred embodiment invention relates to the HCC up-regulated the LOC5.pr hypothetical polypeptide (SEQ ID 6) and to the to the nucleic acid LOC5 (SEQ ID 15) coding for the polypeptide. cDNA corresponding to this mRNA has been identified in cDNA libraries from several human tissues including liver (information from SOURCE database as described above) but the sequence has not previously been reported to be up-regulated in disorders according to the invention, in particular in HCC. Expression of this mRNA is elevated 5-fold relative to non-diseased liver in 71% of the HCC cases profiled. Similar analysis reveals elevated expression of this mRNA in FNH and in a majority of cirrhotic livers subjected to this cDNA microarray expression profiling procedure. The mRNA is expressed in other human gastrointestinal tract carcinomas but only in brain and bone marrow of the 17 non-diseased human tissues examined. LOC5.pr (SEQ ID 6) is a predicted 30 kDa polypeptide (Accession number NP_060917.1 in the GenBank database). The presence of this polypeptide has not been described in any cell or tissue.

No function has been described for this predicted polypeptide and no conserved domains are revealed from a search with the CDD domain algorithm. These results show that the strongly upregulated expression of the LOC5 cDNA sequence is highly specific for disorders according to the invention, especially in HCC, FNH and in a majority of cirrhotic livers. Therefore the LOC5.pr polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of in HCC, FNH, and a majority of cirrhotic livers. With regard to the treatment it is preferred to carry out the treatment such that the expression of the LOC5.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the LOC5.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the LOC5.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the LOC5.pr polypeptide or an antibody fragment thereof which block the activity of the LOC5.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this LOC5.pr polypeptide and/or LOC5 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In a further preferred embodiment the invention relates to the SEC14L2 cDNA (SEQ ID 16) encoding the SEC14L2.pr polypeptide (SEQ ID No. 7) according to the invention. The expression of SEC14L2 mRNA, has been described in many tissues but elevation of this message or the encoded polypeptide has not been previously reported in disorders according to the invention in particular not in liver disorders or cancer. SEC14L2.pr (SEQ ID 7) is a human homologue of the yeast sec polypeptide 14. Although implicated in the yeast secretory pathway, a clear function for this polypeptide or its homologues has not been described in any species. This human sequence has also been suggested to bind to tocopherol and it has been predicted that this polypeptide is involved in squalene transfer, cholesterol biosynthesis or more generally in intracellular transport (Zimmer et al., 2000, J. Biol. Chem. 275:25672-25680). Expression of this polypeptide sequence has not

been reported in human cells or tissues. The polypeptide sequence includes possible G-polypeptide binding and phosphotidylinositol transfer domains and a consensus CRAL_TRIO domain. The latter has been implicated in vitamin binding via the cis-retinal CRAL motif. The mRNA encoding this polypeptide is elevated and average of 5.14-fold or greater relative to non-diseased liver in 71% of HCC samples, in all FNH disease samples profiled, but not in adenoma in only one-half of cirrhosis samples. Expression of the mRNA encoding this polypeptide has been detected in kidney and colon carcinoma and in the normal pancreas but not in other normal tissues examined (Figure 25). These results show that the strongly upregulated expression of the SEC14L2 cDNA sequence is highly specific for disorders according to the invention, especially in HCC and FNH. Therefore the SEC14L2.pr polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC, FNH and preferably also in cirrhosis. With regard to the treatment it is preferred to carry out the treatment such that the expression of the SEC14L2.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the SEC14L2.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the SEC14L2.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the SEC14L2.pr polypeptide or an antibody fragment thereof which block the activity of the SEC14L2.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this SEC14L2.pr polypeptide and/or SEC14L2 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders, and/or other epithelial cancers.

In a further preferred embodiment the invention relates to the a nucleic acid (SEQ ID 17) coding for the SSP29.pr or APRIL polypeptide, which have been described in many tissues and tumors. The gene encoding this putative tumor necrosis family member has not previously been reported to be expressed at elevated levels in disorders according to the invention, in particular in HCC. Furthermore the invention

relates to the silver stainable 29 kDa polypeptide (SSP29.pr; SEQ ID 8) which is encoded by the nucleic acid (SEQ ID 17) according to the invention. The polypeptide has been identified as a leucine rich secreted polypeptide, likely belonging the TNF cytokine family. It is also known as APRIL (acidic polypeptide rich in leucines) and contains leucine rich repeats (LRRs) near the N-terminus that may be involved in antigen-mediated cellular responses. (Zhu et al., 1997, Biochem. Mol. Biol. Int. 42:927-935; Mencinger et al., 1998, Biochim. Biophys. Acta 1395: 176-180). Expression of the SSP29.pr polypeptide has not been reported in human cells or tissues. The mRNA encoding this polypeptide is elevated an average of 3.77-fold relative to non-diseased liver in 17 of 21 HCCs profiled. Surprisingly, the level of the mRNA encoding this polypeptide is 30-fold higher in cirrhosis caused by copper toxicity than in a pool of non-diseased liver. mRNA levels are marginally elevated in other liver disorders profiled relative to non-diseased liver and this mRNA is otherwise detected only infrequently in the normal and diseased tissues subjected here to expression profiling. These results show that the strongly upregulated expression of the SSP29 cDNA sequence is highly specific for disorders according to the invention, especially in HCC, and certain types of cirrhosis disease. Therefore the SSP29.pr polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC and cirrhosis. With regard to the treatment it is preferred to carry out the treatment such that the expression of the SSP29.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the SSP29.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the SSP29.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the SSP29.pr polypeptide or an antibody fragment thereof which block the activity of the SSP29.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this SSP29.pr polypeptide and/or SSP29 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders, and/or other epithelial cancers.

In another preferred embodiment the invention relates to the HS16 nucleic acid (SEQ ID 18). cDNA clones corresponding to this message have been identified in several tissues including adenocarcinoma of the colon but neither this mRNA nor the encoded polypeptide (HS16.pr, SEQ ID 9) have been previously implicated in disorders according to the invention, in particular in liver disorders or in HCC. The invention further relates to the polypeptide encoding for the HS16.pr is a predicted polypeptide of 16.7 kDa (SEQ ID 9; Accession number NP_057223 in the GenBank database). The presence of the polypeptide has not been described in any cell or tissue and its function has not been described nor are functional domains identified with the CDD algorithm. mRNA encoding this polypeptide is elevated at least 2.8-fold or higher in 8 of the HCCs examined and by nearly 2-fold in an additional 4 HCC samples examined, all relative to non-diseased liver. These results show that the strongly upregulated expression of the HS16 cDNA sequence is highly specific for disorders according to the invention, especially in HCC. Therefore the HS16.pr polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of HCC. With regard to the treatment it is preferred to carry out the treatment such that the expression of the HS16.pr polypeptide or of the nucleic acid encoding the polypeptide is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the nucleic acid encoding the HS16.pr polypeptide. Alternatively the treatment may be carried out such that the activity of the HS16.pr polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the HS16.pr polypeptide or an antibody fragment thereof which block the activity of the HS16.pr polypeptide to a patient in need of such treatment. Compared to the state of the art, this HS16.pr polypeptide and/or HS16 nucleic acid surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disordersand/or other epithelial cancers.

In another preferred embodiment the invention relates to the IK3 nucleic acid (SEQ ID 19) which is a highly deregulated mRNA associated with HCC but without a clear open reading frame to encode a corresponding polypeptide. Surpri-

singly the sequence from this mRNA is represented at much higher levels in HCC than in normal human liver. The over-expressed sequence identified here in HCC corresponds to a fetal brain cDNA in the GenBank database (AL049338). Otherwise there is no information regarding expression of this gene in normal or diseased tissues. This sequence may be extended to identify a corresponding polypeptide encoding open reading frame. Alternatively, the provided database sequence may include an error(s) that account for the lack of an open reading frame. Yet another alternative is that the encoded polypeptide may result from one of the small open reading frames in this sequence. Even further, this RNA may be not translated into polypeptide but may have functional (e.g., regulatory) properties itself. This mRNA is elevated an average of 3.81-fold or more relative to non-diseased liver in 12 of the 21 HCC samples profiled (57%). IK3 is also elevated 2-fold or more relative to non-diseased liver in 3 of 4 FNH examined, in adenoma and in 5 of the 6 cirrhosis samples examined. These results show that the strongly upregulated expression of the IK3 cDNA sequence is highly specific for disorders according to the invention, especially in HCC, FNH, adenoma and cirrhosis. Therefore the IK3 polypeptide and/or the encoding nucleic acid can be utilized for the diagnosis, prevention and treatment of disorders according to the invention, in particular for the diagnosis of in HCC, FNH, adenoma and cirrhosis. With regard to the treatment it is preferred to carry out the treatment such that the expression of the polypeptide encoded by the IK3 or of the IK3 nucleic acid is reduced and/or inhibited, for example by administering antisense oligonucleotides or RNA interference molecules that specifically interact with the IK3 nucleic acid. Alternatively the treatment may be carried out such that the activity of the IK3 polypeptide is reduced and/or inhibited, for example by administering an antibody directed against the IK3 polypeptide or an antibody fragment thereof which block the activity of the IK3 polypeptide to a patient in need of such treatment. Compared to the state of the art, this IK3 nucleic acid surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

The Accession numbers of the polypeptides according to the invention and their cDNAs are shown in Figure 1.

In another preferred embodiment of the invention the nucleic acid according to the invention can be used for the construction of antisense oligonucleotides (Zheng and Kemeny, 1995, Clin. Exp. Immunol. 100: 380-2; Nellen and Lichtenstein, 1993, Trends Biochem. Sci. 18: 419-23; Stein, 1992, Leukemia 6: 967-74) and/or ribozymes (Amarzguioui, et al. 1998, Cell. Mol. Life Sci. 54: 1175-202; Vaish et al., 1998, Nucleic Acids Res. 26: 5237-42; Persidis, 1997, Nat. Biotechnol. 15: 921-2; Couture and Stinchcomb, 1996, Trends Genet. 12: 510-5) and/or small interfering double stranded RNAs (Elbashir et al., 2001, Nature 411: 494-98; Brummelkamp et al., 2002, Science 296:550-553). In further preferred embodiments of the invention, the stability of the nucleic acid according to the invention can be decreased and/or the translation of the nucleic acid according to the invention inhibited by using RNA interference molecules (oligonucleotides). Thus, for example, the expression of the corresponding genes in cells can be decreased both *in vivo* and *in vitro* . Oligonucleotides can therefore be suitable as therapeutics. This strategy is also suitable, for example, for liver cells, in particular if the antisense oligonucleotides are complexed with liposomes. For use as a probe or as an " antisense" oligonucleotide, a single-stranded DNA or RNA is preferred.

In a preferred embodiment a nucleic acid according to the invention has been prepared by recombinant methods, by screening a library or isolation from a sample obtained from a patient or a subject. In another preferred embodiment of the invention the nucleic acid according to the invention has been prepared synthetically. Thus, the nucleic acid according to the invention can be synthesized, for example, chemically with the aid of the DNA sequences described in SEQ ID No. 10 to SEQ ID No. 19 and/or with the aid of the protein sequences described in SEQ ID No.1 to SEQ ID No. 9 with reference to the genetic code, e.g. according to the phosphotriester method (see, for example, Uhlmann and Peyman, 1990, Chemical Reviews 90:543-584).

In another preferred embodiment, the invention relates to a nucleic acid according to the invention or a nucleic acid which is a non-functional mutant variant the nucleic acid or a nucleic acid having a sequence complementary to one of the afo-

rementioned nucleic acids, which has been modified by attachment of chemical moieties to the nucleic acid to stabilize it against degradation, so that a high concentration of the nucleic acid is maintained in the cell over a long period (Beigelman et al., 1995, Nucleic Acids Res. 23: 3989-94; Dudycz, 1995, WO 95/11910; Macadam et al., 1998, WO 98/37240; Reese et al., 1997, WO 97/29116). Typically, such a stabilization can be obtained by the introduction of one or more internucleotide phosphorus groups or by the introduction of one or more non-phosphorus internucleotides.

Preferred suitable modified internucleotides are summarized in Uhlmann and Peymann (1990 Chem. Rev. 90, 544; see also Beigelman et al., 1995 Nucleic Acids Res. 23: 3989-94; Dudycz, 1995, WO 95/11910; Macadam et al., 1998, WO 98/37240; Reese et al., 1997, WO 97/29116).

In a further embodiment the invention relates to a vector containing a nucleic acid according to the invention or a nucleic acid which is a non-functional mutant variant the nucleic acid or a nucleic acid having a sequence complementary to one the aforementioned nucleic acids. Preferably the vector is a knock-out gene construct, a plasmid, a shuttle vector, a phagemid, a cosmid, a viral vector, an expression vector and/or a vector applicable in gene therapy.

The preparation of such constructs is generally known to the person skilled in the art.

An "expression vector" within the meaning of the present invention, preferably comprises at least one promoter or enhancer, i.e. at least one regulatory element comprising at least one translation initiation signal, at least one of the nucleic acids according to the invention or a nucleic acid which is a non-functional mutant variant the nucleic acid or a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, one translation termination signal, a transcription termination signal, and a polyadenylation signal for the expression in eukaryotes.

For the expression of the gene concerned, in general a double-stranded DNA is preferred, the DNA region coding for the polypeptide being particularly preferred. In the case of eukaryotes this region begins with the first start codon (ATG) lying in a Kozak sequence (Kozak, 1987, Nucleic. Acids Res. 15: 8125-48) up to the next stop codon (TAG, TGA or TAA), which lies in the same reading frame to the ATG. In the case of prokaryotes this region begins with the first AUG (or GUG) after a Shine-Dalgarno sequence and ends with the next stop codon (TAA, TAG or TGA), which lies in the same reading frame to the ATG.

Differentially expressed genes in HCC can contain cancer or liver or liver cancer gene-specific regulatory sequences. These non-transcribed sequences, found in the tissue- or disease-specific gene may be used to drive tissue- or disease-specific expression of included therapeutic and/or tumor cell-cytotoxic genes. These regulatory sequences may be used for liver cancer specific expression of a nucleic acid according to the invention or a nucleic acid which is a non-functional mutant variant the nucleic acid or a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids. The screening and construction of such regulatory a sequences is generally known to the person skilled in the art.

Suitable expression vectors can be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors are, for expression in *E. coli*, e.g. the vectors pGEM or pUC derivatives, examples of eukaryotic expression vectors are for expression in *Saccharomyces cerevisiae*, e.g. the vectors p426Met25 or p426GAL1 (Mumberg et al. (1994) Nucl. Acids Res., 22, 5767-5768), for expression in insect cells, e.g. *Baculovirus* vectors such as disclosed in EP-B1-0 127 839 or EP-B1-0 549 721, and for expression in mammalian cells, e.g. the vectors Rc/CMV and Rc/RSV or SV40 vectors, which are all generally obtainable. Specific vectors for production of RNA interference following transfection, such as the pSUPER vector (Brummelkamp et al., 2002, Science 296:550-553) are also included.

In general, the expression vectors also contain promoters suitable for the respective cell, such as, for example, the trp promoter for expression in *E. coli* (see, for example, EP-B1-0 154 133), the MET 25, GAL 1 or ADH2 promoter for expression in yeast (Russel et al. (1983), J. Biol. Chem. 258, 2674-2682; Mumberg, supra), the Baculovirus polyhedrin promoter, for expression in insect cells (see, for example, EP-B1-0 127 839). For expression in mammalian cells, for example, suitable promoters are those which allow a constitutive, regulatable, tissue-specific, cell-cycle-specific or metabolically specific expression in eukaryotic cells. Regulatory elements according to the present invention preferably are promoters, activator sequences, enhancers, silencers and/or repressor sequences.

Examples of suitable regulatory elements which make possible constitutive expression in eukaryotes preferably are promoters which are recognized by the RNA polymerase III or viral promoters, CMV enhancer, CMV promoter, SV40 promoter or LTR promoters, e.g. from MMTV (mouse mammary tumor virus; Lee et al. (1981) Nature 214, 228-232) and further viral promoter and activator sequences, derived from, for example, adeno- and adeno-like viruses, HBV, HCV, HSV, HPV, EBV, HTLV or HIV.

Examples of regulatory elements which make possible regulated expression in eukaryotes are the tetracycline operator in combination with a corresponding repressor (Gossen et al., 1994, Curr. Opin. Biotechnol. 5: 516-20).

Translation initiation signals, translation termination signals, transcription termination signals, and polyadenylation signals are generally known to the person skilled in the art and can be readily obtained from commercial laboratory suppliers.

Preferably, the expression of the genes relevant for liver disorders and/or epithelial cancer takes place under the control of tissue-specific promoters, for example, under the control of liver-specific promoters such as albumin, alpha fetoprotein, apolipoprotein AI, alpha-1 antitrypsin, and the complement C5 and C8A genes (Schrem et al., 2002, Pharmacol. Rev. 54 129-58; Pontoglio et al., 2001, J. Expt.

Med. 194:1683-1689). The regulatory sequences associated with genes highly deregulated in HCC as described herein also provide a preferable method for specific gene expression in these disorders.

Further examples of regulatory elements which make tissue-specific expression in eukaryotes possible are promoters or activator sequences from promoters or enhancers of those genes which code for proteins which are only expressed in certain cell types.

Examples of regulatory elements which make possible metabolically specific expression in eukaryotes are promoters which are regulated by hypoxia, by oxidative stress, by glucose deficiency, by phosphate concentration or by heat shock.

Examples of regulatory elements which make cell cycle-specific expression in eukaryotes possible are promoters of the following genes: cdc25A, cdc25B, cdc25C, cyclin A, cyclin E, cdc2, E2F-1 to E2F-5, B-myb or DHFR (Zwicker J. and Müller R.,1997, Trends Genet. 13: 3-6). The use of cell cycle regulated promoters is particularly preferred in cases, in which expression of the polypeptides or nucleic acids according to the invention is to be restricted to proliferating cells.

In order to make possible the introduction of nucleic acids as described above or a nucleic acid which is a non-functional mutant variant the nucleic acid and thus the expression of the polypeptide in a eu- or prokaryotic cell by transfection, transformation or infection, the nucleic acid can be present as a plasmid, as part of a viral or non-viral vector. Suitable viral vectors here are particularly: baculoviruses, vaccinia viruses, adenoviruses, adeno-associated viruses, retroviruses and herpesviruses. Suitable non-viral vectors here are particularly: virosomes, liposomes, cationic lipids, or polylysine-conjugated DNA or naked DNA.

Plasmids, shuttle vectors, phagemids, and cosmids suitable for use according to the invention are also known to the person skilled in the art and are generally obtainable from commercial laboratory suppliers.

Examples of vectors applicable in gene therapy are virus vectors, for example adenovirus vectors, retroviral vectors or vectors based on replicons of RNA viruses (Lindemann et al., 1997, Mol. Med. 3: 466-76; Springer et al., 1998, Mol. Cell. 2: 549-58, Khromykh, 2000, Curr. Opin. Mol Ther. 2:555-569). Eukaryotic expression vectors are suitable in isolated form for gene therapy use, as naked DNA can penetrate, for example, into liver cells upon local application or via the blood supply.

Compared to the state of the art, this fusion construct surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders, and/or other epithelial cancers.

In another aspect the invention furthermore relates to a cell containing a nucleic acid according to the invention. Preferably the cell is transformed with a vector according to the invention. The cell preferably contains a nucleic acid wherein the nucleic acid is either a non-functional mutant variant of a nucleic acid according to the invention or a variant thereof. In particular the cell contains vector containing a nucleic acid wherein the nucleic acid is a non-functional mutant variant of a nucleic acid according to the invention or a variant thereof. Perferably the cell contains a nucleic acid coding for a nucleic acid having a sequence complementary to a nucleic acid according to the invention or a variant thereof. Moreover the cell preferably contains a vector containing a nucleic acid coding for an antibody according to the invention or a fragment of the antibody. The cell according to the invention, may for example be a liver cell, containing at least one of the aforementioned nucleic acids or a cell which is transformed using one of the above described vectors. Cells can be either prokaryotic or eukaryotic cells, heterologous or autologous cells. Examples of prokaryotic cells are *E. coli* and examples of eukaryotic

cells include primary hepatocytes cells, hepatocytes cell lines such as HepG2 cells, yeast cells, for example *Saccharomyces cerevisiae* or insect cells.

Compared to the state of the art, the cell according to the invention surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

In a preferred embodiment of the invention the cell is a transgenic embryonic non-human stem cell which comprises at least one nucleic acid according to the invention, at least one vector, at least one knock-out gene construct and/or at least one expression vector as described above.

Processes for the transformation of cells and/or stem cells are well known to a person skilled in the art and include, for example, electroporation or microinjection.

In another aspect the invention relates to the provision of a transgenic non-human mammal containing a compound selected from the group consisting of a nucleic acid according to the invention, a nucleic acid which is a non-functional mutant variant the nucleic acid, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, one of the aformementioned nucleic acids in the form of a vector, of a knock-down or knock-out gene construct, and of an expression vector.

Transgenic animals in general show a tissue-specifically increased expression of the nucleic acids and/or polypeptides and can be used for the analysis of liver disorders and/or epithelial cancers, such as for example HCC, and for development and evaluation of therapeutic strategies for such disorders. Transgenic animals may further be employed in the production of polypeptides according to the invention. The polypeptide produced by the animal may for example be enriched in a body

fluid of the animal. The polypeptides according to the invention may for example be isolatable from a body fluid such as the milk.

Compared to the state of the art, this transgenic non-human mammal surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive analysis and/or diagnosis of a liver disorders and/or other epithelial cancers.

Processes for the preparation of transgenic animals, in particular of transgenic mice, are likewise known to the person skilled in the art from DE 196 25 049 and US 4,736,866; US 5,625,122; US 5,698,765; US 5,583,278 and US 5,750,825 and include transgenic animals which can be produced, for example, by means of direct injection of expression vectors according to the invention into embryos or spermatocytes or by injection of the expression vectors into the pronucleus of the fertilized ovum or by means of the transfection of expression vectors into embryonic stem cells or by nuclear transfer into appropriate recipient cells (Polites and Pinkert, DNA Microinjection and Transgenic Animal Production, page 15 to 68 in Pinkert, 1994, Transgenic animal technology: a laboratory handbook, Academic Press, London, UK; Houdebine, 1997, Harwood Academic Publishers, Amsterdam, The Netherlands; Doetschman, Gene Transfer in Embryonic Stem Cells, page 115 to 146 in Pinkert, 1994, supra; Wood, Retrovirus-Mediated Gene Transfer, page 147 to 176 in Pinkert, 1994, supra; Monastersky, Gene Transfer Technology; Alternative Techniques and Applications, page 177 to 220 in Pinkert, 1994, supra).

If the above described nucleic acids are integrated into so-called "targeting vectors" or "knock-out" gene constructs (Pinkert, 1994, supra), it is possible after transfection of embryonic stem cells and homologous recombination, for example, to generate knock-out mice which, in general, as heterozygous mice, show decreased expression of the nucleic acid, while homozygous mice no longer exhibit expression of the nucleic acid. The animals thus produced can also be used for the analysis of liver disorders, such as for example HCC, and/or epithelial cancers.

Knock-out gene constructs are known to the person skilled in the art, for example, from the US patents 5,625,122; US 5,698,765; US 5,583,278 and US 5,750,825.

In a further aspect the invention relates to an antibody or a fragment thereof is provided, wherein the antibody or antibody fragment is directed against a polypeptide according to the invention, a funtional variant thereof or against a nucleic acid coding for the polypeptide or a variant thereof.

Compared to the state of the art, these antibody or a fragment thereof surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis and/or improved, sustained and/or more effective treatment of the liver disorders and/or other epithelial cancers.

The term "antibody" or "antibody fragment" is understood according to the present invention as also meaning antibodies or antigen-binding parts thereof prepared by genetic engineering and optionally modified, such as, for example, chimeric antibodies, humanized antibodies, multifunctional antibodies, bi- or oligospecific antibodies, single-stranded antibodies, F(ab) or $F(ab)_2$ fragments (see, for example, EP-B1-0 368 684, US 4,816,567, US 4,816,397, WO 88/01649, WO 93/06213, WO 98/24884). The antibodies according to the invention can for example be used for diagnosis, prevention and/or treatment of disorders according to the invention such as liver disorders, for example HCC, and/or epithelial cancers.

The invention further relates to a method for producing an antibody or antibody fragment, preferably a polyclonal or monoclonal antibody, specific for the polypeptides and the polypeptides encoded by the nucleic acids according to the invention for example for the diagnosis and/or prevention and/or treatment of disorders according to the invention. The process is carried out according to methods generally known to the person skilled in the art by immunizing a mammal, for example a rabbit, with a nucleic acid according to the invention, or with a polypeptide according to the invention or parts thereof having at least 6 amino acid length, preferably having at least 8 amino acid length, in particular having at least 12 amino

acid length, if appropriate in the presence of, for example, Freund's adjuvant and/or aluminum hydroxide gels (see, for example, Harlow and Lane, 1998, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Press, New York, USA, Chapter 5, pp. 53-135). The polyclonal antibodies formed in the animal as a result of an immunological reaction can then be easily isolated from the blood according to generally known methods and purified, for example, by means of column chromatography. Monoclonal antibodies can be produced, for example, according to the known method of Winter & Milstein (Winter and Milstein, 1991, Nature 349:293-299).

The present invention further relates to an antibody or antibody fragments directed against a polypeptide described above and reacts specifically with the polypeptides described above, where the above-mentioned parts of the polypeptide are either immunogenic themselves or can be rendered immunogenic by coupling to suitable carriers, such as, for example, bovine serum albumin or keyhole limpet hemocyanin to increase in their immunogenicity. This antibody is either polyclonal or monoclonal, preferably it is a monoclonal antibody.

Still further, the present invention relates to the generation and/or production of an antibody or antibody fragment specific for the polypeptide according to the invention from a recombinant antibody expression library, such as for example described by Knappik et al. (2000, J. Molec. Biol. 296:57-86) or by Chadd and Chamow (2001 Curr. Opin. Biotechnol. 12:188-94).

In another embodiment of the invention, it is provided an array, wherein the array contains at least two compounds selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid according to SEQ ID No. 19, a non-functional mutant variant the nucleic acid and an antibody or an antibody fragment directed against the polypeptide. Alternatively, the array may contain at least 1 component according to the invention in combination with previously described components implicated in neoplastic or metabolic liver disorders or epithelial cancers.

Within the meaning of the invention the term "array" refers to a solid-phase or gel-like carrier upon which at least two compounds are attached or bound in one-, two- or three-dimensional arrangement. Such arrays are generally known to the person skilled in the art and are typically generated on glass microscope slides, specially coated glass slides such as polycation-, nitrocellulose- or biotin- coated slides, cover slips, and membranes such as for example membranes based on nitrocellulose or nylon.

The aforementioned arrays include bound polypeptides according to the invention or functional variants thereof or nucleic acids coding for the polypeptides or variants thereof, fusion proteins according to the invention or antibodies or antibody fragments directed against polypeptides according to the invention or functional variants thereof or cells expressing polypeptides according to the invention or functional variants thereof or at least two cells expressing at least one nucleic acid according to the invention or variants thereof. Nucleic acids coding for these or variants thereof can also be part of an array. Such arrays can be employed for analysis and/or diagnosis of liver disorders, preferably of HCC, and/or epithelial cancer.

The invention further relates to a method of producing arrays according to the invention, wherein at least two compounds according to the invention are bound to a carrier material.

Methods of producing such arrays, for example based on solid-phase chemistry and photo-labile protective groups are generally known (US 5,744,305). Such arrays can also brought into contact with substances or a substance libraries and tested for interaction, for example for binding or change of conformation.

The invention further relates to a process for preparing an array immobilized on a support material for analysis and/or diagnosis of disorders according to the inventi-

on such as a liver disorder, preferably of HCC, in which at least two nucleic acid, at least two polypeptide or at least two antibody or antibody fragment, and/or at least two cell, or at least one of the aforementioned components in combination with other components relevant to neoplastic and metabolic liver disorders or epithelial cancers, as described above, is used for preparation. The arrays produced by such process can be employed for the diagnosis of disorders according to the invention.

In another aspect the invention relates to a diagnostic contains at least one compound selected from the group consisting of a polypeptide according to the invention or a functional variant thereof, a nucleic acid encoding the polypeptide, preferably a nucleic acid according to SEQ ID No. 10 - 18 or or a nucleic acid coding for the SEQ ID No. 19 or a variant of one of the aformementioned nucleic acids, and an antibody or an antibody fragment according to the invention, combined or together with suitable additives or auxiliaries.

Compared to the state of the art, this diagnostic surprisingly allow improved, more sensitive, earlier, faster, and/or non-invasive diagnosis of liver disordersand/or other epithelial cancers.

Within the meaning of the invention "suitable additives" or "auxiliaries" are generally known to the person skilled in the art and comprise, for example, physiological saline solution, demineralized water, gelatin or glycerol-based protein stabilizing reagents. Alternatively, the nucleic acid or polypeptide according to the invention may be lyophilized for stabilization.

In another example a diagnostic kit based on the nucleic acid sequences according to the invention could be generated. Such a kit may be designed specifically to detect cells altered as a result of the described disorders resident in the circulatory system and thereby detectable in serum from test patients. Additional examples of diagnostic kits includes enzyme linked immunosorbent assays (ELISA), radioimmunoassays (RIA), and detection of an immune reaction or specific antibodies to

the polypeptides according to the invention including detection of specific responding immune cells.

In a preferred embodiment the diagnostic according to the invention contains a probe, preferentially a DNA probe.

For example, it is possible according to the present invention to prepare a diagnostic based on the polymerase chain reaction (PCR). Under defined conditions, preferably using primers specific for a nucleic acid according to the invention as a DNA probe PCRs specific for the nucleic acid sequences of the invention will be utilized to monitor both the presence, and especially the amount, of specific nucleic acids according to the invention in a sample isolated from a patient obtained for diagnostic or therapeutic purposes. This opens up a further possibility of obtaining the described nucleic acids, for example by isolation from a suitable gene or cDNA library, for example from a liver disorder-specific or liver specific gene bank, with the aid of a suitable probe (see, for example, J. Sambrook et al., 1989, Molecular Cloning. A Laboratory Manual 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY Chapter 8 pages 8.1 to 8.81, Chapter 9 pages 9.47 to 9.58 and Chapter 10 pages 10.1 to 10.67).

Suitable probes are, for example, DNA or RNA fragments having a length of about 50-1000 nucleotides, preferably having a length of about 10 to about 100 nucleotides, preferably about 100 to about 200 nucleotides, in particular having a length of about 200-500 nucleotides, whose sequence can be derived from the polypeptides according to SEQ ID No. 1 to SEQ ID No. 9 and functional variants thereof and nucleic acids coding for the polypeptides, preferably according to SEQ ID No. 10 to SEQ ID No. 19 and variants thereof.

Alternatively, it is preferably possible with the aid of the derived nucleic acid sequences to synthesize oligonucleotides which are suitable as primers for a polymerase chain reaction. Using this, the nucleic acid described above or parts of this can be amplified and isolated from cDNA, for example HCC-specific cDNA. Suitable

primers are, for example, DNA fragments having a length of about 10 to 100 nucleotides, preferably having a length of about 15 to 50 nucleotides, in particular having a length of 17 to 30 nucleotides, whose sequence can be derived from the polypeptides according to SEQ ID No. 1 to SEQ ID No. 9 or from the nucleic acids according to SEQ ID No. 10 to SEQ ID No. 19. The design and synthesis of such primers is generally known to the person skilled in the art. The primers may additionally contain restriction sites, e.g. suitable for integration of the amplified sequence into vectors, or other adapters or overhang sequences, e.g. having a marker molecule such as a fluorescent marker attached, generally known to the skilled worker.

In another aspect of the invention it is provided a method of diagnosis of a disorder according to the invention, wherein at least one compound selected from the group consisting of a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, and an antibody directed against the polypeptide or antibody fragment thereof, is identified in the sample of a patient and compared with at least one compound of a reference library or of a reference sample.

In a preferred embodiment of the method the disorder of the liver is a disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia.

In a preferred embodiment of the method the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

Compared to the state of the art, this diagnostic surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis of the liver disorders and/or other epithelial cancers.

Preferably the sample is isolated from a patient by non-invasive methods as described above.

For example, serum detection of specific deregulated gene proteins via ELISA assay is one application, alternatively one or a panel of antibodies to deregulated gene products from which a diagnostic score is deduced based on the combinations of, and expression levels of gene products expressed in the diseased tissue or in serum from diseased individuals.

A preferred diagnostic according to the invention contains the described polypeptide or the immunogenic parts thereof described in greater detail above. The polypeptide or the parts thereof, which are preferably bound to a solid phase, e.g. of nitrocellulose or nylon, can be brought into contact *in vitro*, for example, with the body fluid to be investigated, e.g. blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, in order thus to be able to react, for example, with autoimmune antibodies present in e.g. the blood of the patient. The antibody-peptide complex can then be detected, for example, with the aid of labeled antihuman IgG antibodies. The labeling involves, for example, an enzyme, such as peroxidase, which catalyses a color or chemiluminescent reaction. The presence and the amount of autoimmune antibody present can thus be detected easily and rapidly by means of the color.

In addition the diagnostic may be directed to detecting an endogenous antibody or fragment thereof present in the sample isolated from a patient which antibody or fragment thereof is directed against a polypeptide according to the invention. Detection of such autoimmune antibodies may be accomplished by methods generally known to the skilled artisan, e.g. by immunoaffinity assays using polypeptides according to the invention or functional variants thereof or parts thereof as a probe.

Preferably the presence of such autoimmune antibodies is indicative of the patient suffering from a disorder according to the invention.

A further diagnostic, that is subject matter of the present invention, contains the antibodies according to the invention themselves. With the aid of these antibodies, it is possible, for example, to easily and rapidly investigate a tissue sample as to whether the concerned polypeptide according to the invention is present in an increased amount in order to thereby obtain an indication of possible disease including liver disorders, for example HCC. In this case, the antibodies according to the invention are preferably labeled directly, or more commonly for example these are detected with a specific secondary antibody indirectly, such as with an enzyme or fluorescent molecule, as already described above. The specific antibody-peptide complex can thereby be detected easily and rapidly, e.g., by means of an enzymatic color reaction.

In still another aspect of the invention it is provided a method for identifying at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof differentially expressed in a sample isolated from a patient relative to a reference library or a reference sample comprising the following steps:

1. Detecting the expression of at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof in a sample isolated from a patient,
2. Comparing the expression of at least one nucleic acid detected in step 1 with the expression of the same at least one nucleic acid in a reference library or in a reference sample,
3. Identifying this at least one nucleic acid which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample.

Compared to the state of the art the method surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive identification of differentially ex-

pressed nucleic acids according to the invention which provides a useful basis for diagnosing a disorder according to the invention.

Preferably at least 2, at least 3, at least 4 at least 5, at least 6, or at least 7 nucleic acids are identified.

In another preferred embodiment of the method the at least one nucleic acid is detected by PCR based detection or by a hybridization assay.

In another preferred embodiment of the method the expression of the at least one nucleic acid is compared by a method selected from the group consisting of solid-phase based screening methods, hybridization, subtractive hybridization, differential display, and RNase protection assay.

In a further preferred embodiment of the method the sample isolated from the patient is selected from the group consisting of liver tissue, a liver cell, tissue from another organ subject to cancerous transformation, a cell from this organ, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

Preferably the reference sample is isolated from a source selected from a non-diseased sample of the same patient or a non-diseased sample from another subject. The selection of appropriate reference samples is generally known to the person skilled in the art. In particular the reference sample may be selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

In another preferred embodiment of the method, the reference library is an expression library or a data base containing clones or data on non-diseased expression of at least one nucleic acid according to the invention in samples that preferably may be selected from the group consisting of liver tissue, a liver cell, blood, serum,

plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

In another aspect of the invention it is provided a method of diagnosing a liver disorder, and/or another epithelial cancer comprising the following steps:

1. Detecting the expression of at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof in a sample isolated from a patient,
2. Comparing the expression of at least one nucleic acid detected in step 1 with the expression of the same at least one nucleic acid in a reference library or in a reference sample,
3. Identifying this at least one nucleic acid which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample, and
4. Matching the at least one nucleic acid identified in step 3 with at least one nucleic acid differentially expressed in a pathologic reference sample or pathologic reference library,

wherein the matched nucleic acid(s) are indicative of the patient suffering from a liver disorder, and/or other epithelial cancer.

Compared to the state of the art, this method of diagnosing surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis of the liver disorders and/or other epithelial cancers.

Preferably at least 2, at least 3, at least 4 at least 5, at least 6, or at least 7 nucleic acids are identified.

In another preferred embodiment of the method the at least one nucleic acid is detected by PCR based detection or by a hybridization assay.

In another preferred embodiment of the method the expression of the at least one nucleic acid is compared by a method selected from the group consisting of solid-phase based screening methods, hybridization, subtractive hybridization, differential display, and RNase protection assay.

In a further preferred embodiment of the method the sample isolated from the patient is selected from the group consisting of liver tissue, a liver cell, tissue from another organ subject to cancerous transformation, a cell from this organ, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

Preferably the reference sample is isolated from a source selected from a non-diseased sample of the same patient or a non-diseased sample from another subject. The selection of appropriate reference samples is generally known to the person skilled in the art. In particular the reference sample may be selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

In another preferred embodiment of the method of diagnosis, the reference library is an expression library or a data base containing clones or data on non-diseased expression of the at least one nucleic acid according to the invention in samples that preferably may be selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

In another preferred embodiment of the method of diagnosis, the pathologic reference sample is isolated from a diseased sample from another patient. The latter patient having been diagnosed as suffering from the disorder according to the invention which is to be diagnosed. The selection of appropriate pathologic reference samples is generally known to the person skilled in the art. In particular the pathologic reference sample may be selected from the group consisting of liver tissue, a

liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

In another preferred embodiment of the method of diagnosis, the pathologic reference library is a data base containing data on differential expression of the at least one nucleic acid according to the invention in samples isolated from at least one patient, excluding the patient under diagnosis, suffering from the disorder according to the invention to be diagnosed in the inventive method relative to control expression in a reference sample or reference library. The pathologic reference library preferably also relates to a differential expression library containing nucleic acids according to the invention which are differentially expressed in samples isolated from at least one patient, excluding the patient under diagnosis, suffering from the disorder according to the invention to be diagnosed in the inventive method relative to control expression in a reference sample or reference library. The selection of an appropriate pathologic reference library is generally known to the person skilled in the art.

Preferably the liver disorder, is a disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia. In particular the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

Within the meaning of the invention the term "detecting a nucleic acid" refers to a method that preferably uncovers, visualizes, separates or allows recognition of the nucleic acid according to the invention from the background of the other components present in the sample. Such methods are generally known to the person skilled in the art and include in situ hybridization, PCR amplification, gel electrophoresis, northern blots, solid phase array (gene chips) based methods, nuclease protection methods (as described and referenced in Alberts, et al. (2002) The Molecular Biology of the Cell, 4th ed. Garland, New York, USA).

Within the meaning of the invention the term "comparing the expression of at least one nucleic acid detected in step 1 with the expression of the same at least one nucleic acid in a reference library or in a reference sample" refers to a comparison of the expression of the two groups of at least one nucleic acid on a quantitative or qualitative level by means of an experimental procedure such as differential display, subtractive hybridization, RNAse protection assay, or especially DNA chip hybridization. Moreover a comparison of experimental data on the at least one nucleic acid detected in step 1 with the expression of the nucleic acids in a reference library as defined above is also included herein.

The term "Identifying this at least one nucleic acid which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample" within the meaning of the present invention is understood to mean selecting this at least one nucleic acid which is differentially expressed compared to the reference library or the reference samples which fulfills the following criteria: the level of differential expression of the detected at least one nucleic acid compared to the reference library or the reference samples is greater than about 2 fold, preferably greater than about 5 fold, more preferred greater than about 10 fold upregulated.

The term "matching the at least one nucleic acid identified in step 3 with at least one nucleic acid differentially expressed in a pathologic reference sample or pathologic reference library " within the meaning of the invention is understood to mean that this at least one nucleic acid identified in step 3 is compared with at least one nucleic acid differentially expressed in a pathologic reference sample or pathologic reference library. Then this the at least one nucleic acid identified in step 3 that is also differentially expressed in the pathologic reference sample or pathologic reference library is matched, i.e. at least one identical pair is identified and allocated. Since the differential expression of the at least one nucleic acid in the pathologic reference sample or pathologic reference library is indicative of a disorder according to the invention, such correspondence with the differential expression in the sample then indicates that the patient suffers from that disorder.

Preferably the sample is isolated from a patient by non-invasive or preferably minimally invasive methods such as described above, including venupuncture.

The methods of diagnosing according to the invention allows early detection of a liver disorder and/or an epitherlial cancer, and/or non-invasive diagnosis of the disorder, based on an essentially concordant expression pattern of the nucleic acids according to the invention detected in the samples isolated from an animal and/or a human patient suffering from a liver disorder and/or an epithelial cancer relative to a reference sample or relative to a reference library. The method has the additional advantage that it also provides additional and novel diagnostic parameters to characterize different subtypes of liver disorders, such as for example subtypes of HCC.

The term "essentially concordant expression pattern" of the nucleic acids according to the invention refers to a pattern of expression that is essentially reproducible from patient to patient or subject to subject, provided that the patients or subjects compared are in the same or comparable pathological condition or healthy condition, respectively.

In still another aspect of the invention it is provided a method for identifying at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof differentially expressed in a sample isolated from a patient relative to a reference library or a reference sample comprising the following steps:

1. Detecting the expression of at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof in a sample isolated from a patient,

2. Comparing the expression of at least one polypeptide detected in step 1 with the expression of the same at least one polypeptide in a reference library or in a reference sample,

3. Identifying this at least one polypeptide which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample.

Compared to the state of the art, this method surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive identification of differentially expressed polypeptides according to the invention which provides a useful basis for diagnosing a disorder according to the invention.

Preferably at least 2, at least 3, at least 4, at least 5, at least 6, or at least 7 polypeptides are identified.

Preferably the sample is isolated from a patient by non-invasive or minimally invasive methods such as described above, including venupuncture.

In another embodiment of the method the sample is a sample as defined further above. Preferably the reference sample is a reference sample as defined above.

In another preferred embodiment of the method, the reference library is an expression library or a data base containing clones or data on non-diseased expression of the at least one polypeptide according to the invention in samples that preferably may be selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces. Such data bases are generated as a result of the cDNA microarray expression analysis according to the invention and are known to persons skilled in the art. Further reference libraries useable according to the invention have been described above.

In another aspect of the invention it is provided a method of diagnosing a liver disorder and/or an epithelial cancer comprising the following steps:

1. Detecting the expression of at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof in a sample isolated from a patient,

2. Comparing the expression of at least one polypeptide detected in step 1 with the expression of the same at least one polypeptide in a reference library or in a reference sample,

3. Identifying this at least one polypeptide which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample, and

4. Matching the at least one polypeptide identified in step 3 with at least one polypeptide differentially expressed in a pathologic reference sample or pathologic reference library,

wherein the matched polypeptide(s) are indicative of the patient suffering from a liver disorder and/or an epithelial cancer.

Compared to the state of the art, this method of diagnosing surprisingly allows improved, more sensitive, earlier, faster, and/or non-invasive diagnosis of the liver disorders and/or other epithelial cancers.

Preferably at least 2, at least 3, at least 4, at least 5, at least 6, or at least 7 polypeptides are identified.

Within the meaning of the invention the term "detecting a polypeptide" refers to a method that preferably uncovers, visualizes, separates and/or allows recognition of the polypeptide according to the invention from the background of the other components present in the sample. Such methods are generally known to the person skilled in the art and includes gel electrophoresis, chromatographic techniques, immunoblot analysis, immunohistochemistry, enzyme based immunoassay, mass spectroscopy, high pressure liquid chromatography, surface plasmon resonance, and/or antibody and protein arrays as described above (Ausubel, F.A. et al., eds., 1990, Current Protocols in Molecular Biology. Greene Publishing and Wiley-

Interscience, New York, USA, Chapter 10; Myszka and Rich 2000, Pharm. Sci. Technol. Today 3:310-317). Preferably proteins and polypeptides are prepared from the sample by disruption of the cells with physical sheering or ultrasonic means, for example. Protein is denatured and stabilized with reducing agent treatment and heating and the protein is size fractionated on electrophoretic polyacrylamide gels.

Within the meaning of the invention the term "comparing the expression of at least one polypeptide detected in step 1 with the expression of the same at least one polypeptide in a reference library or in a reference sample " refers to a comparison of the expression of the two groups of at least one polypeptide on a quantitative and/or qualitative level by means of an experimental procedure such as two dimensional gel electrophoresis, chromatographic separation techniques, immunoblot analysis, surface plasmon resonance, immunohistochemistry, and enzyme based immunoassay. In two dimensional gel electrophoresis all peptides are first resolved according to isoelectric point in the first electrophoretic dimension and then by size according to methods well known to persons experienced in the art. Moreover a comparison of experimental data on the at least one polypeptide detected in step 1 with the expression of the polypeptide in a reference library as defined above is also included herein.

The term "Identifying this at least one polypeptide which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample" within the meaning of the present invention is understood to mean selecting this at least one polypeptide which is differentially expressed compared to the reference library or the reference samples which fulfills the following criteria: the level of differential expression of the detected at least one polypeptide compared to the reference library or the reference samples is greater than about 2 fold, preferably greater than about 5 fold, more preferred greater than about 10 fold upregulated.

The term "matching the at least one polypeptide identified in step 3 with at least one polypeptide differentially expressed in a pathologic reference sample or patho-

logic reference library " within the meaning of the invention is understood to mean that this at least one polypeptide identified in step 3 is compared with at least one polypeptide differentially expressed in a pathologic reference sample or pathologic reference library. Then this the at least one polypeptide identified in step 3 that is also differentially expressed in the pathologic reference sample or pathologic reference library is matched, i.e. at least one identical pair is identified and allocated. Since the differential expression of the at least one polypeptide in the pathologic reference sample or pathologic reference library is indicative of a disorder according to the invention, such correspondence with the differential expression in the sample then indicates that the patient suffers from that disorder.

Preferably the sample is isolated from a patient by non-invasive or minimally invasive methods such as described above, including venupuncture.

In another embodiment of the method the sample is a sample as defined further above. Preferably the reference sample is a reference sample as defined above.

In another preferred embodiment of the method of diagnosis, the reference library is an expression library or a database containing clones or data on non-diseased expression of the at least one polypeptide according to the invention in samples that preferably may be selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

An example of a data base according to the invention and further experimental reference libraries useable according to the invention have been described above.

In another preferred embodiment of the method of diagnosis, the pathologic reference sample is a pathologic reference sample as has been defined above.

In another preferred embodiment of the method of diagnosis, the pathologic reference library is a data base containing data on differential expression of the at least one polypeptide according to the invention in samples isolated from at least one patient, excluding the patient under diagnosis, suffering from the disorder according to the invention to be diagnosed in the inventive method relative to control expression in a reference sample or reference library. The pathologic reference library also relates to a differential expression library containing polypeptides according to the invention which are differentially expressed in samples isolated from at least one patient, excluding the patient under diagnosis, suffering from the disorder according to the invention to be diagnosed in the inventive method relative to control expression in a reference sample or reference library. The selection of an appropriate pathologic reference library is generally known to the person skilled in the art.

Preferably the liver disorder, is a disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia. In particular the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

The methods of diagnosing according to the invention allows early detection of a liver disorder and/or epithelial cancer, and/or non-invasive diagnosis of the disorder, based on an essentially concordant expression pattern of the polypeptides according to the invention detected in the samples isolated from an animal and/or a human patient suffering from a liver disorder and/or epithelial cancer relative to a reference sample or relative to a reference library. The method has the additional advantage that it also provides additional and novel diagnostic parameters to characterize different subtypes of liver disorders , such as for example subtypes of HCC.

The term "essentially concordant expression pattern" of the polypeptides according to the invention refers to a pattern of expression that is essentially reproducible from patient to patient or subject to subject, provided that the patients or subjects compared are in the same or comparable pathological condition or healthy condition, respectively.

In another aspect of the invention it is provided a pharmaceutical composition containing at least one compound selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against the polypeptide, a fragment of the antibody, a vector containing a nucleic acid coding for the antibody, a cell containing the vector containing a nucleic acid coding for the antibody, and a cell containing the vector containing a nucleic acid coding for the antibody fragment, combined or together with suitable additives or auxiliaries. In a preferred embodiment the pharmaceutical composition contains at least one cell according to the invention, combined or together with suitable additives or auxiliaries.

When compared to the state of the art of therapy of liver disorders, and/or other epithelial cancers the pharmaceutical composition according to the invention surprisingly provide an improved, sustained and/or more effective treatment.

A pharmaceutical composition in the sense of the invention encompasses medicaments which can be used for preventing and/or treating a liver disorders and/or epithelial cancer. The pharmaceutical composition includes, for instance, a stabilized recombinant antibody that has been produced by expression of specific antibody gene fragments in a cellular system, preferably a eukaryotic system. A recombi-

nant antibody therapeutic for instance, is delivered by injection into the diseased liver region or into the venous or arterial vascular systems or into the hepatic portal system. The injections can be repeated at regular intervals to achieve therapeutic efficacy. Therapeutics according this invention may also be employed in combinations with other chemical, antibody, or any other therapeutic application to improve efficacy.

The present invention also relates to a process producing a pharmaceutical composition for the treatment and/or prevention of disorders according to the invention, for example, HCC, in which at least one component selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide or a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against the polypeptide, a fragment of the antibody, a vector containing a nucleic acid coding for the antibody, a cell containing the vector containing a nucleic acid coding for the antibody, and a cell containing the vector containing a nucleic acid coding for the antibody fragment, is combined or together with suitable additives.

The present invention furthermore relates to a pharmaceutical composition produced by this process for the treatment and/or prevention of liver disorders and/or epithelial cancers, for example, HCC, which contains at least one component selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide or a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell

containing the vector, an antibody directed against the polypeptide, a fragment of the antibody, a vector containing a nucleic acid coding for the antibody, a cell containing the vector containing a nucleic acid coding for the antibody, and a cell containing the vector containing a nucleic acid coding for the antibody fragment, , if appropriate together with suitable additives and auxiliaries. The invention furthermore relates to the use of this pharmaceutical composition for the prevention and/or treatment of liver disorders, for example, HCC and/or epithelial cancer.

Preferably the pharmaceutical composition is employed for the treatment of a liver disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia. In particular the phramaceutical composition is employed for the treatment of an epithelial cancer which is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

Therapy can also be carried out in a conventional manner generally known to the person skilled in the art, e.g. by means of oral application or via intravenous injection of the pharmaceutical compositions according to the invention. It is thus possible to administer the pharmaceutical composition containing the suitable additives or auxiliaries, such as, for example, physiological saline solution, demineralized water, stabilizers, proteinase inhibitors.

A therapy based on the use of cells, which express at least one polypeptide according to the invention, of functional variants thereof or nucleic acids coding for the polypeptide or a nucleic acid according to the SEQ ID No. 19, or variants thereof can be achieved by using autologous or heterologous cells. Preferred cells comprise liver cells, for example primary cultures of liver cells, liver populating stem or progenitor cells, or blood cells. The cells can be applied to the tissue, preferably to the blood or injected into the liver, with suitable carrier material. Such therapy is preferably based on the notion that upon expression and/or release of a

polypeptide according to the invention the polypeptide stimulates an immune response in the patient in need of the treatment.

Preferably the therapeutical approach is directed toward inhibiting the function and/or expression of at least one polypeptides according to the invention and/or the function and/or expression of at least one nucleic acids according to the invention. Such inhibition of the expression and/or function preferably reduces the expression and/or function of the targeted nucleic acid/polypeptide significantly. The inhibition of the expression and/or function preferably abolishes the expression and/or functioning of the targeted nucleic acid/polypeptide. Such reduction or abolished expression and/or functioning of the targeted nucleic acid/polypeptide can be determined using conventional assays for determining the expression and/or functioning of a polypeptide/nucleic acid generally known to the person skilled in the art. In particular such assays for determining the function comprise methods for comparing the biological activity of the targeted nucleic acid/polypeptide before and after administration of the pharmaceutical composition. Preferably such assays for determining the expression comprise methods for comparing the level of expression of the targeted nucleic acid/polypeptide before and after administration of the pharmaceutical composition.

Such therapy is preferably accomplished by the use of a nucleic acid having a sequence complementary to one of nucleic acids according to the invention, i.e. an antisense molecule or a RNA interference molecule which reduces or abolishes the translation of transcribed nucleic acids according to the invention and thereby inhibits the function and/or expression of the targeted nucleic acid/polypeptide. Preferably such nucleic acid having a complementary sequence may be employed in the form of a vector or a cell containing such nucleic acid. On the polypeptide level the therapy may in particular be carried out by the use of an antibody or an antibody fragment directed against a polypeptide according to the invention. The antibody or antibody fragment may be administered directly to the patient or preferably the nucleic acid encoding the antibody is contained in a vector which is preferably contained in a cell. The cell or vector may then be administered to the patient in need of such treatment.

When compared to the state of the art of therapy of liver disorders, and/or other epithelial cancers the method of treating according to the invention surprisingly provide an improved, sustained and/or more effective treatment.

The invention further relates to a method of treating a patient suffering from of a liver disorder, wherein at least one component selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide or a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against the polypeptide, a fragment of the antibody, a vector containing a nucleic acid coding for the antibody, a cell containing the vector containing a nucleic acid coding for the antibody, and a cell containing the vector containing a nucleic acid coding for the antibody fragment, optionally combined or together with suitable additives and/or auxilaries, is administered to the patient in need of a the treatment in a therapeutically effective amount.

Preferably the method of treatment is directed to a liver disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia. In particular the method of treatment is directed to an epithelial cancer which is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

Methods of administering such compounds or cells have been described in detail above.

The term "therapeutically effective amount" refers to the administration of an amount of the compound to the patient that results in an "effective treatment" as defined above. Determination of the therapeutically effective amount of the compound(s) is generally known to the person skilled in the art.

Such methods of treating allow effective treatment of a liver disorder and/or epithelial cancers as described above.

In another aspect of the invention it is provided a method of stimulating an immune response a patient suffering from a liver disorder and/or an epithelial cancer to a polypeptide according to the invention or a functional variant thereof, wherein at least one component selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, and a cell containing the vector, is administered to the patient in need of such treatment in an amount effective to stimulate the immune response in the patient.

When compared to the state of the art of therapy of liver disorders, and/or other epithelial cancers the method of stimulating an immune response according to the invention surprisingly provide an improved, sustained and/or more effective immunization.

In another aspect of the invention it is provided a method of preventing a patient from developing a liver disorder and/or an epithelial cancer, wherein at least one component selected from the group consisting of a polypeptide according to the invention, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a vector containing one of the afore-

mentioned nucleic acids, a cell containing one of the aforementioned nucleic acids, and a cell containing the vector, is administered to the patient in need of such preventive treatment in a therapeutically effective amount.

When compared to the state of the art of therapy of liver disorders, and/or other epithelial cancers the method of preventing according to the invention surprisingly provide an improved, sustained and/or more effective preventive measure.

Preferably the method of preventing and/or method of stimulating an immune response is directed to a liver disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia. In particular, preferably the method of preventing and/or method of stimulating an immune response is directed to an epithelial cancer which is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

In a further aspect the invention relates to a method of identifying at least one pharmacologically active compound comprising the following steps:

1. providing at least one polypeptide according to the SEQ ID No. 2 or a functional variant thereof,
2. contacting the at least one polypeptide, with at least one putative pharmacologically active compound,
3. assaying the interaction of the at least one putative pharmacologically active compound and the at least one polypeptide,
4. identifying the at least one pharmacologically active compound among the assayed at least one putative pharmacologically active compound by selecting those assayed at least one putative pharmacologically active compound which directly or indirectly functionally interact with the at least one polypeptide.

Preferably the at least one polypeptide is provided in a form selected from the group of the at least one polypeptide is attached to a column, the at least one polypeptide is attached to an array, the at least one polypeptide is is contained in an electrophoresis gel, the at least one polypeptide is attached to a membrane, and the at least one polypeptide is expressed by a cell.

It is preferred to assay the interaction by a method selected from the group of enzyme and fluorescence based cellular reporter assays in which interaction of the at least one pharmacological substance with a recombinant fusion protein including the at least one polypeptide is detected. The interaction may preferably also be assayed by surface plasmon resonance, HPLC and mass spectroscopy. Preferably the direct or indirect functional interaction is selected from the group consisting of induction of the expression of the at least one polypeptide, inhibition of the expression of the at least one polypeptide, activation of the function of the the at least one polypeptide, inhibition of the function of the the at least one polypeptide.

The term "pharmacologically active substance" in the sense of the present invention is understood as meaning all those molecules, compounds and/or compositions and substance mixtures which can interact under suitable conditions with the polypeptide according to the SEQ ID No. 2 or functional variants thereof, if appropriate together with suitable additives and/or auxiliaries. Possible pharmacologically active substances are simple chemical (organic or inorganic) molecules or compounds, but can also include peptides, proteins or complexes thereof. Examples of pharmacologically active substances are organic molecules that are derived from libraries of compounds that have been analyzed for their pharmacological activity. On account of their interaction, the pharmacologically active substances can influence the expression and/or function(s) of the polypeptide *in vivo* or *in vitro* or alternatively only bind to the polypeptides described above or enter into other interactions of covalent or non-covalent manner with them.

A suitable test system which can be used in accordance with the invention is based on identifying interactions with the two hybrid system (Fields and Sternglanz, 1994, Trends in Genetics, 10, 286-292; Colas and Brent, 1998 TIBTECH, 16, 355-363). In this test system, cells are transformed with expression vectors which express fusion proteins which consist of at least one polypeptide according to the invention and a DNA-binding domain of a transcription factor such as Gal4 or LexA. The transformed cells also contain a reporter gene whose promoter contains binding sites for the corresponding DNA-binding domain. By means of transforming a further expression vector, which expresses a second fusion protein consisting of a known or unknown polypeptide and an activation domain, for example from Gal4 or herpes simplex virus VP16, the expression of the reporter gene can be greatly increased if the second fusion protein interacts with the investigated polypeptide according to the invention. This increase in expression can be used for identifying new interacting partners, for example by preparing a cDNA library from e.g., liver tissue, or diseased liver tissue for the purpose of constructing the second fusion protein. In a preferred embodiment, the interaction partner is an inhibitor of the polypeptide according to the SEQ ID No. 2 or functional variants thereof. This test system can also be used for screening substances which inhibit an interaction between the polypeptide according to the invention and an interacting partner. Such substances decrease the expression of the reporter gene in cells which are expressing fusion proteins of the polypeptide according to the invention and the interacting partner (Vidal and Endoh, 1999, Trends in Biotechnology, 17: 374-81). In this way, it is possible to rapidly identify novel active compounds which can be employed for the therapy of and/or prevention of liver disorders and/or epithelial cancer.

Assays for identifying pharmacologically active substances which exert an influence on the expression of proteins are well known to the skilled person (see, for example, Sivaraja et al., 2001, US 6,183,956). Thus, cells which express the polypeptide according to the SEQ ID No. 2 or functional variants thereof, can be cultured as a test system for analyzing gene expression *in vitro*, with preference being given to liver cells. Gene expression is analyzed, for example, at the level of the mRNA or of the proteins using methods generally known to the person skilled in

the art. In this connection, the quantity of polypeptide according to the SEQ ID No. 2 or mRNA present after adding one or more putative pharmacologically active substances to the cell culture is measured and compared with the corresponding quantity in a control culture. This is done, for example, with the aid of an antibody specifically directed against the polypeptide according to the SEQ ID No. 2 or a functional variant thereof, which can be used to detect the polypeptide present in the lysate of the cells. The amount of expressed polypeptide can be quantified by methods generally known to the person skilled in the art using, for example, an ELISA or a Western blot. In this connection, it is possible to carry out the analysis as a high-throughput method and to analyze a very large number of substances for their suitability as modulators of the expression of the polypeptide according to the SEQ ID No. 2 (Sivaraja et al., 2001, US 6,183,956). In this connection, the substances to be analyzed can be taken from substance libraries (see, e.g. DE19816414, DE19619373) which can contain several thousand substances which are frequently very heterogeneous.

The invention will now be further illustrated below with the aid of the figures and examples, representing preferred embodiments and features of the invention without the invention being restricted hereto.

Description of the tables, figures and sequences:

Figure 1: nucleic acids and polypeptides according to the invention and their respective SEQ ID Nos. and Accession numbers from the GenBank.

Figure 2: depicts the OBcl1.pr polypeptide sequence of SEQ ID No. 1.

Figure 3: depicts the OBcl5.pr polypeptide sequence of SEQ ID No. 2.

Figure 4: depicts the IK2.pr polypeptide sequence of SEQ ID No. 3.

Figure 5: depicts the IK5.pr polypeptide sequence of SEQ ID No. 4.

Figure 6: depicts the DAP3.pr polypeptide sequence of SEQ ID No. 5.

Figure 7: depicts the LOC5.pr polypeptide sequence of SEQ ID No. 6.

Figure 8: depicts the SEC14L2.pr polypeptide sequence of SEQ ID No. 7.

Figure 9: depicts the SSP29.pr polypeptide sequence of SEQ ID No. 8.

Figure 10: depicts the HS16.pr polypeptide sequence of SEQ ID No. 9.

Figure 11: depicts proposed full length OBcl1 cDNA sequence (SEQ ID 10), sequence from deregulated SSH cDNA clone given in small capital letters. Sequence compiled includes GenBank entries AL050205, 2312bp - 3' end

(including SSH sequence); AF131755, 2135bp ; XM_113703 2130bp (w/open reading frame (ORF); AY004310 1615bp (also w/ ORF).

Figure 12: depicts the OBcl5 nucleic acid sequence of SEQ ID No. 11.

Figure 13: depicts the IK2 nucleic acid sequence of SEQ ID No. 12.

Figure 14: depicts the IK5 nucleic acid sequence of SEQ ID No. 13.

Figure 15: depicts the DAP3 nucleic acid sequence of SEQ ID No. 14.

Figure 16: depicts the proposed full length LOC5 mRNA sequence for deregulated cDNA clone (SEQ ID 15). The sequence corresponds to GenBank Accession no. NM_018447.

Figure 17: depicts the SEC14L2 nucleic acid sequence of SEQ ID No. 16

Figure 18: depicts the SSP29 nucleic acid sequence of SEQ ID No. 17

Figure 19: depicts the HS16 nucleic acid sequence of SEQ ID No. 18

Figure 20: depicts the IK3 nucleic acid sequence of SEQ ID No. 19

Figure 21: cDNA microarray nucleic acid expression level ratios. The expression levels relative to a non-diseased liver reference sample of sequences according to the invention assessed in tissues from human liver disorders, including HCC. The values are ratios of expression levels between diseased and non-diseased liver samples, obtained from competitive hybridization to custom-made cDNA microarrays. HCC = hepatocellular carcinoma samples; FNH = focal nodular hyperplasia samples; Cirrh = cirrhosis samples; Copper tox. = cirrhosis with copper toxicity etiology; Adenoma = adenoma; Non-dis.liver = Non-diseased liver; mean = average of ratio values for each group (HCC, FNH and Cirrh).

Figure 22: Summary of cDNA microarray nucleic acid expression values. The median expression level value from experimental (diseased) and control (non-diseased) tissues for each nucleic acid according to the invention is provided. The Wilcoxon rank sum two-sided test was applied to these data (paired flag = 'false') to demonstrate the difference between groups (Hollander & Wolfe, 1973, Nonpara-

metric statistical inference. New York: John Wiley & Sons, pgs. 27-33, 68-75; Bauer, D.F., 1972, J. Amer. Statistical Assoc. 67: 687-690). The expression values typically do not fit a normal distribution so averaging the values may be misleading. However, analysis of the median values demonstrates significant differences in most cases between experimental and reference values, particularly in the large data sets. Expt. median = median value for experimental (diseased) tissues; Expt. iqr = experimental value interquartile range (+/- 25% of median value); Contr. median = median value for control (non-diseased) tissue samples; Contr. iqr = control value interquartile range (+/- 25% of median value); p value = value resulting from statistical evaluation of the probability that the experimental and control values are significantly different. Ninety-five percent confidence in difference significance provides a p value of 0.05.

Figure 23:

Summary boxplot of expression values in HCC versus non-diseased liver cDNA microarray experiments. The box plot is a graphical representation of all expression value ratios with the median value indicated by a horizontal line. The extent of each box indicates the interquartile range (iqr, +/- 25% of the median value); error bars indicate 1.5 times the iqr. Ratios that do not fall within this range are indicated as circles. For each nucleic acid according to the invention, elevated ex-

pression is apparent in HCC (at least 2 fold high than the non-diseased liver values; the y-axis indicates the fold induction of gene expression in experimental vs. control tissues). Expression values are consistently elevated in a similar ratio except for OBcl5 (SEQ ID No. 11) where the differences in expression between patient and control samples is most dramatically different.

Figure 24: Comparison of nucleic acid expression values in non-neoplastic liver diseases and liver cancer. For each nucleic acid according to the invention a p value is provided for the difference in the median experimental expression values for comparisons between FNH, liver cirrhosis (Cirrh.) and HCC samples. For each nucleic acid and comparison a value of less than or equal to 0.05 indicates a significant difference in expression values between the disease groups. Significance was assessed with the Wilcoxon rank sum test. Statistically significant differences in expression are evident between disease groups. For example, the expression values for IK2 are significantly different in all three comparisons (p values less than 0.05). The FNH sample group is small and displayed a large distribution of values which likely accounts for fewer significant differences in comparisons with this group.

Figure 25: RT-PCR analysis of nucleic acid expression in human non-diseased and disease tissues. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed with primers specific for each deregulated nucleic acid in each tissue listed. All tissues employed were diagnostically confirmed prior to utilization for RNA (and cDNA) preparation. The "+" symbol indicates that the gene is expressed in the tissue, the "-" indicates that this gene was not detected in cDNA from this RNA sample; and a blank box indicates that the analysis was not performed for that gene and tissue combination. The patient's age and sex and provided. Additional sample information includes the tumor staging value (T = tumor size), as well as the tumor grading score (G = tumor cell differentiation); large numbers indicate larger and less well-differentiated tumors, respectively. The positive control for tissue cDNA is amplification from the glyceraldehyde phosphate dehydrogenase mRNA (GAPDH).

Figure 26: Representative RT-PCR data demonstrating expression of the nucleic acids according to the invention in independent HCC samples and controls. Amplification of the 'housekeeping' gene GAPDH was included in parallel reactions with each cDNA template to control for cDNA quality. 5 to 10% of the RT-PCR reaction products subjected to 30-40 PCR cycles were loaded onto this agarose gel. Puri-

fied DNA from the HCC library pool is included as a positive control (C) for each nucleic acid according to the invention. Two independent HCC samples (H) were included in this analysis together with one non-diseased liver sample (N) for representative nucleic acids according to the invention. M = molecular mass marker (100 bp ladder).

Figure 27: Verification of differential gene expression by RNA blot. Independent evaluation of RNA samples from a pool of 3 non-diseased livers (L) and from 2 HCC tissues (H) verifies the increased expression of OBcl1 (SEQ ID No. 10) and OBcl5 (SEQ ID No. 11) in this image of an RNA blot autoradiogram as indicated on the figure. The results from the antisense strand probe (top; specific signal) and the corresponding sense strand probe negative control (bottom) demonstrate the specificity of hybridization with the antisense probe.

SEQ ID No. 1 to SEQ ID No. 9 refer to the human sequences of polypeptide according to the invention from.

SEQ ID No. 10 to SEQ ID No. 19 refer to the human cDNA sequences according to the invention.

Further embodiments and preferred features of the invention are provided below.

1. An isolated polypeptide comprising a sequence according to SEQ ID No. 2 or a functional variant thereof.

2. A fusion protein, wherein the fusion protein contains a polypeptide according to the embodiment 1.

3. An isolated nucleic acid encoding a polypeptide according to the embodiment 1 or a variant thereof.

4. A nucleic acid according to the embodiment 3, wherein the nucleic acid is a single-stranded or double-stranded RNA.

5. A nucleic acid according to the embodiment 3, wherein the nucleic acid is a nucleic acid according to SEQ ID No.11.

6. A vector, wherein the vector contains a nucleic acid selected from the group consisting of a nucleic acid according to the embodiment 3, a nucleic acid coding for a polypeptide according to the SEQ ID No. 1-9, a nucleic acid according to the SEQ ID No. 19.

7. A vector according to the embodiment 6, wherein the vector is selected from the group consisting of a knock-out gene construct, a plasmid, a shuttle vector, a phagemid, a cosmid, a viral vector, and an expression vector.

8. A cell, wherein the cell contains a nucleic acid according to the embodiment 3.

9. A cell, wherein the cell is transformed with a vector according to the embodiment 6.

10. A cell according to the embodiment 9, wherein the cell is a transgenic embryonic non-human stem cell.

11. A transgenic non-human mammal, wherein the transgenic non-human mammal contains the nucleic acid according to the embodiment 3.

12. An antibody or an antibody fragment thereof, wherein the antibody is directed against a polypeptide according to the embodiment 1 or against a nucleic acid coding for the polypeptide.

13. A nucleic acid which comprises a nucleic acid having a sequence complementary to a nucleic acid according to the embodiment 3 or a non-functional mutant variant of the nucleic acid according to the embodiment 3.

14. A nucleic acid according to the embodiment 13, wherein nucleic acid having a complementary sequence is an antisense molecule or an RNA interference molecule.

15. A vector, wherein the vector contains a nucleic acid according to the embodiment 13.

16. A vector according to the embodiment 15, wherein the vector is selected from the group consisting of a plasmid, a shuttle vector, a phagemid, a cosmid, a viral vector, and an expression vector.

17. A cell, wherein the cell contains a nucleic acid according to the embodiment 13.

18. A cell, wherein the cell is transformed with a vector according to the embodiment 15.

19. A diagnostic, wherein the diagnostic contains at least one compound selected from the group consisting of a polypeptide according to the embodiment 1, a polypeptide according to SEQ ID No. 1 to 9, a nucleic acid encoding one of the aforementioned polypeptides, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, an antibody

directed against the polypeptide, and a fragment of the antibody, combined or together with suitable additives or auxiliaries.

20. A diagnostic according to the embodiment 19, wherein the nucleic acid is a probe, preferentially a DNA probe.

21. A pharmaceutical composition, wherein the pharmaceutical composition contains at least one component selected from the group consisting of a polypeptide according to the embodiment 1, a polypeptide according to SEQ ID No. 1 to 9, a functional variant of the polypeptide, a nucleic acid encoding one of the aforementioned polypeptides, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against the polypeptide, a fragment of the antibody, a vector containing a nucleic acid coding for the antibody, a cell containing the vector containing a nucleic acid coding for the antibody, and a cell containing the vector containing a nucleic acid coding for the antibody fragment, combined or together with suitable additives or auxiliaries.

22. A pharmaceutical composition according to the embodiment 21, wherein the nucleic acid having a complementary sequence is an antisense molecule or an RNA interference molecule.

23. A method of diagnosis of a liver disorder or an epithelial cancer, wherein at least one compound selected from the group consisting of a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid according to SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, an antibody directed against the polypeptide, and a fragment of the

antibody, is identified in the sample of a patient and compared with at least one compound of a reference library or of a reference sample.

24. A method according to one of the embodiment 23, wherein the liver disorders disorder, is a disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia.

25. A method according to one of the embodiments 23, wherein the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

26. A method of treating a patient suffering from a liver disorder or an epithelial cancer, wherein at least one component selected from the group consisting of a polypeptide according SEQ ID No. 1-9, a functional variant of the polypeptide, a nucleic acid encoding the polypeptide, a nucleic acid encoding the functional variant, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, a cell containing the vector, an antibody directed against one of the aforementioned polypeptides, an antibody directed against a functional variant of one of the aforementioned polypeptides, a fragment of one of the aforementioned antibodies, a vector containing a nucleic acid coding for one of the aforementioned antibodies, a vector containing a nucleic acid coding for one of the aforementioned antibody fragments, a cell containing the vector containing a nucleic acid coding for one of the aforementioned antibodies, and a cell containing the vector containing a nucleic acid coding for one of the aforementioned antibody fragments, is administered to the patient in need of a the treatment in a therapeutically effective amount.

27. A method of treating according to the embodiment 26, wherein the nucleic acid having a complementary sequence is an antisense molecule or an RNA interference molecule.

28. A method of treating according to the embodiment 27, wherein the RNA interference molecule is administered in the form of a double stranded RNA or a vector expressing the double stranded RNA

29. The method according to the embodiment 28, wherein the RNA interference molecule has a size range selected from the group consisting of from 15 to 30 nucleotides, from 20 - 25 nucleotides, and from 21-23 nucleotides.

30. A method according to one of the embodiment 26, wherein the liver disorder, is a disorder selected from the group consisting of cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia.

31. A method according to one of the embodiments 26, wherein the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

32. A method of stimulating an immune response a patient suffering from a liver disorder or an epithelial cancer to a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof, wherein at least one component selected from the group consisting of a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, and a cell contai-

ning the vector, is administered to the patient in need of such treatment in an amount effective to stimulate the immune response in the patient.

33. A method for identifying at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof differentially expressed in a sample isolated from a patient relative to a reference library or a reference sample comprising the following steps:

1. Detecting the expression of at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof in a sample isolated from a patient,

2. Comparing the expression of at least one nucleic acid detected in step 1 with the expression of the same at least one nucleic acid in a reference library or in a reference sample,

3. Identifying this at least one nucleic acid which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample.

34. A method of diagnosing a liver disorder or an epithelial cancer comprising the following steps:

1. Detecting the expression of at least one nucleic acid according to SEQ ID No. 10 to SEQ ID No. 19 or a variant thereof in a sample isolated from a patient,

2. Comparing the expression of at least one nucleic acid detected in step 1 with the expression of the same at least one nucleic acid in a reference library or in a reference sample,

3. Identifying this at least one nucleic acid which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample, and

4. Matching the at least one nucleic acid identified in step 3 with at least one nucleic acid differentially expressed in a pathologic reference sample or pathologic reference library,

wherein the matched nucleic acid(s) are indicative of the patient suffering from a liver disorder, or an epithelial cancer.

35. A method according to the embodiment 34, wherein at least 2, at least 3, at least 4 at least 5, at least 6, or at least 7 nucleic acids are identified.

36. A method according to the embodiment 34, wherein the at least one nucleic acid is detected by PCR based detection or by a hybridization assay.

37. A method according to the embodiment 34, wherein the expression of the at least one nucleic acid is compared by a method selected from the group consisting of solid-phase based screening methods, hybridization, subtractive hybridization, differential display, and RNase protection assay.

38. A method according to the embodiment 34, wherein the sample isolated from the patient is selected from the group consisting of liver tissue, a liver cell, tissue from another organ subject to cancerous transformation, a cell from this organ, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

39. A method according to the embodiment 34, wherein the reference sample is isolated from a source selected from a non-diseased sample of the same patient, and a non-diseased sample from another subject.

40. A method according to the embodiment 34, wherein the reference sample is selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

41. A method according to the embodiment 34, wherein the reference library is an expression library or a data base containing clones or data on liver disorder-specific expression of the at least one nucleic acid.

42. A method according to one of the embodiments 34, wherein the pathologic reference sample is isolated from a source selected from a diseased sample from another patient suffering from a liver disorder, and epithelial cancer.

43. A method according to one of the embodiments 34, wherein the pathologic reference library is a data base containing data on differential expression of the at least one nucleic acid according to the invention in samples isolated from another patient, suffering from a liver disorder or epithelial cancer relative to control expression in a reference sample or reference library.

44. A method according to one of the embodiment 34, wherein the liver disorders, is a disorder selected from the group consisting of hepatocellular carcinoma, benign liver neoplasms, and cirrhosis.

45. A method according to one of the embodiments 34, wherein the epithelial cancer is an adenocarcinoma of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin and the breast.

46. A method for identifying at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof differentially expressed in a sample isolated from a patient relative to a reference library or a reference sample comprising the following steps:

1. Detecting the expression of at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or a functional variant thereof in a sample isolated from a patient,

2. Comparing the expression of at least one polypeptide detected in step 1 with the expression of the same at least one polypeptide in a reference library or in a reference sample,

3. Identifying this at least one polypeptide which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample.

47. A method of diagnosing a liver disorder or epithelial cancers comprising the following steps:

1. Detecting the expression of at least one polypeptide according to SEQ ID No. 1 to SEQ ID No. 9 or functional variants thereof in a sample isolated from a patient,

2. Comparing the expression of at least one polypeptide detected in step 1 with the expression of the same at least one polypeptide in a reference library or in a reference sample,

3. Identifying this at least one polypeptide which is differentially expressed in the sample isolated from the patient compared to the reference library or the reference sample, and

4. Matching the at least one polypeptide identified in step 3 with at least one polypeptide differentially expressed in a pathologic reference sample or pathologic reference library,

wherein the matched polypeptide(s) are indicative of the patient suffering from a liver disorder, or an epithelial cancer.

48. A method according to the embodiment 47, wherein at least 2, at least 3, at least 4, at least 5, at least 6, or at least 7 polypeptides are identified.

49. A method according to the embodiment 47, wherein the polypeptides are detected by a method selected from the group consisting of gel electrophoresis, chromatographic techniques, immunoblot analysis, immunohistochemistry, enzyme based immunoassay, surface plasmon resonance, HPLC, mass spectroscopy, immunohistochemistry, and enzyme based immunoassay.

50. A method according to the embodiment 47, wherein the polypeptides are compared by a method selected from the group consisting of two dimensional gel electrophoresis, chromatographic separation techniques, immunoblot analysis, surface plasmon resonance, immunohistochemistry, and enzyme based immunoassay.

51. A method according to the embodiment 47, wherein the sample isolated from a patient is selected from the group consisting of liver tissue, a liver cell, tissue from another organ subject to cancerous transformation, a cell from this organ, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

52. A method according to the embodiment 47, wherein the reference sample is isolated is from a source selected from a non-diseased sample of the same patient, and a non-diseased sample from another subject.

53. A method according to the embodiment 47 wherein the reference sample is selected from the group consisting of liver tissue, a liver cell, blood, serum, plasma, ascitic fluid, pleural effusion, cerebral spinal fluid, saliva, urine, semen, and feces.

54. A method according to the embodiment 47, wherein the reference library is an expression library or a data base containing clones or data on liver disorder-specific expression of the at least one polypeptide.

55. A method according to one of the embodiments 47, wherein the pathologic reference sample is isolated from a source selected from a diseased sample from another patient suffering from a liver disorder, and epithelial cancer.

56. A method according to one of the embodiment 47, wherein the pathologic reference library is a data base containing data on differential expression of the at least one polypeptide according to the invention in samples isolated from another patient, suffering from a liver disorder or epithelial cancer relative to control expression in a reference sample or reference library.

57. A method according to one of the embodiment 47, wherein the liver disorders is a disorder selected from the group consisting of hepatocellular carcinoma, benign liver neoplasms, and cirrhosis,

58. A method according to the embodiment 47, wherein the epithelial cancer is an adenocarcinoma of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

59. A method of preventing a patient from developing a liver disorder or an epithelial cancer, wherein at least one component selected from the group consisting of a polypeptide according to the sequence of SEQ ID No. 1 to SEQ ID No. 9, a functional variant thereof, a nucleic acid encoding the polypeptide, a nucleic acid coding for the SEQ ID No. 19, a variant of one of the aforementioned nucleic acids, a nucleic acid having a sequence complementary to one of the aforementioned nucleic acids, a nucleic acid which is a non-functional mutant variant of one of the aforementioned nucleic acids, a vector containing one of the aforementioned nucleic acids, a cell containing one of the aforementioned nucleic acids, and a cell containing the vector, is administered to the patient in need of such preventive treatment in a therapeutically effective amount.

60. A method of identifying at least one pharmacologically active compound comprising the following steps:

1. providing at least one polypeptide according to the SEQ ID No. 2 or a functional variant thereof
2. contacting the at least one polypeptide, with at least one putative pharmacologically active compound,
3. assaying the interaction of the at least one putative pharmacologically active compound and the at least one polypeptide,
4. identifying the at least one pharmacologically active compound among the assayed at least one putative pharmacologically active compound by selecting those assayed at least one putative pharmacologically active compound which directly or indirectly functionally interact with the at least one polypeptide.

61. The method according to the embodiment 60, wherein the at least one polypeptide is provided in a form selected from the group of the at least one polypeptide is attached to a column, the at least one polypeptide is attached to an array, the at least one polypeptide is is contained in an electrophoresis gel, the at least one polypeptide is attached to a membrane, the at least one polypeptide is attached to a column, and the at least one polypeptide is expressed by a cell.

62. The method according to the embodiment 60, wherein the interaction is assayed by a method selected from the group of enzyme and fluorescence based cellular reporter assays in which interaction of the at least one pharmacological substance with a recombinant fusion protein including the at least one polypeptide is detected. The interaction may preferably also be assayed by surface plasmon resonance, HPLC and mass spectroscopy.

63. The method according to the embodiment 60, wherein the direct or indirect functional interaction is selected from the group consisting of induction of the expression of the at least one polypeptide, inhibition of the expression of the at least one polypeptide, activation of the function of the the at least one polypeptide, inhibition of the function of the the at least one polypeptide.

Examples

Example 1. Identification of nucleic acids deregulated in liver disorders

A unique approach employed in this invention utilized discrete, pathologist-confirmed liver cancer pathologies for production of disease specific cDNA libraries enriched in genes specifically up- and down-regulated in HCC compared with a pool of non-neoplastic human livers. The library is a genome-wide representation of deregulated gene expression in HCC and therefore includes all potential HCC deregulated genes. Repetitive hybridization to these library clones with labeled expressed nucleic acids from many additional discrete, pathologist-confirmed liver cancer samples (HCCs) and non-malignant liver lesions indicated nucleic acids highly deregulated in HCC. The surprising finding is that this approach provided

deregulated nucleic acids that had not previously been identified as well as many deregulated nucleic acids that were not before associated with HCC, the elevated expression of which can also be associated with other neoplasms. These HCC deregulated genes are the subject of this invention.

The screening and verification strategy is already inventive *per se* owing to the elaborate and defined choice of parameters. Identification of differentially expressed genes according to the invention, relied upon histopathologically distinguished liver disease tissue for comparison of gene expression changes in disorders of the human liver. Non-diseased reference liver samples for the experiments was also diagnostically confirmed and pooled from 3 independent samples to reduce detection of false positives resulting from inter-individual variations.

Example 2. Preparation of HCC subtracted cDNA libraries.

RNA was isolated from three pathologist-confirmed HCC tumor samples and from three pathologist-confirmed non-diseased human liver samples using the TRIZOL reagent (LifeTechnologies) according to standard methods (Chomczynski & Sacchi, 1987, Anal. Biochem. 162:156-159). The tissues used for the generation of cDNA libraries was from patients that provided specific informed consent for utilization of this material for research purposes, including commercial research. mRNA was converted to double stranded cDNA with reverse transcriptase and DNA polymerase as described in the instructions provided in the "PCR select cDNA subtraction kit" from Clontech Laboratories. To enrich for cDNAs specifically increased and decreased in HCC, cDNAs expressed in common and at similar levels in the reference liver pool and in HCC were removed by subtractive suppressive hybridization (SSH) according to the instructions provided in this kit and as described by Diatchenko et al. (1996, Proc. Natl. Acad. Sci. USA 93:6025-6030). The SSH steps were performed in both directions (subtracting non-diseased liver cDNAs from HCC cDNAs and subtracting HCC cDNAs from non-diseased liver cDNAs) so the resulting cDNA molecules represent nucleic acid sequences both up- and down-regulated in HCC but do not represent those that are not differen-

tially expressed. In addition a normalized but not subtracted HCC cDNA library was generated to better represent rare mRNA transcripts in HCC tissues. These cDNAs were separately cloned into the pCRII vector (Invitrogen) by ligation into this plasmid followed by electrophoretic transformation into *E. coli* XL-1-Blue electroporation-competent cells (Stratagene). The cloning was carried out as described by the supplier of the vector and competent cells. Cloned differentially expressed cDNAs were plated onto selective (ampicillin) media to isolate individual clones. 960 clones were isolated from each SSH library and 576 clones isolated from the normalized HCC library and cultures established in 96-well microtiter plates. Together these 2500 cDNA clones provide a unique representation of mRNA expression specific for human HCC tissue.

Example 3. Preparation and hybridization of HCC cDNA microarrays.

1 ml cultures of the SSH cDNA library clones described above were established and the cDNA inserts amplified by PCR with primers specific to the vector sequence flanking the cDNA inserts. The M13 forward (5'-GTAAAACGACGGCCAG-3';SEQ ID NO. 20) and M13 reverse primers (5'-CAGGAAACAGCTATGAC-3'; SEQ ID NO. 21) were employed for the PCR amplification of clone inserts. Fifty microliters of the bacterial cultures were heat denatured at 95°C for 10 minutes, debris removed by centrifugation, and 2 µl of the supernatant included in a standard PCR [1X Amplitaq PCR buffer, 2.5 mM $MgCl_2$, 37.5 nM each primer, 0.5 mM each of dATP, dCTP, dGTP and dTTP and 1.5 units Amplitaq DNA polymerase (Applied Biosystems)]. Reaction conditions were 95°C for 5 minutes followed by 35 cycles of: 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds; then followed by 72°C for 7 minutes and then cooled to 4°C. Amplification of cDNA inserts was confirmed by electrophoresis of a 5% of the PCR on a 1% agarose gel containing 0.4 µg/ml ethidium bromide and run in 1X Tris Acetate EDTA (TAE; 40mM Tris-acetate, 1mM EDTA, pH 7.5) buffer . Each of the SSH clone amplified insert sequences were affixed to sialinized glass microscope slides (GAPS Corning) using a GeneticMicrosystems (now Affymetrix) 417 cDNA arrayer robot to generate custom HCC cDNA microarrays. The protocol for spotting the cDNA inserts to the slides was according to that published

by Hedge et al. (2000, Biotechniques 29:548-560) except that PCR products were spotted directly from the PCR microtiter plates without purification or adjustment of the cDNA buffer. In addition to the SSH cDNA clone inserts, numerous control DNAs were spotted onto the microarrays as controls for hybridization reactions. Further, approximately 2000 publicly available cDNA clones corresponding to genes previously reported to be involved in cancer, especially liver cancer, were purchased from the German Genome Research Center (RZPD), expanded, amplified and spotted onto these microarrays as described above.

For preparation of hybridization probes, 20 micrograms of RNA from additional pathology-confirmed liver disorders and from the same quantity of pooled non-diseased liver RNA was converted to cy5-fluorescence-labeled and cy3-fluorescence-labeled cDNA, respectively (cy5-CTP and cy3-CTP, Pharmacia) using reverse transcriptase according to the standard methods (Hedge et al., 2000, Biotechniques 29: 548-560). Using this protocol, these labeled cDNAs were competitively hybridized to the HCC microarrays. Following prehybridization at 42°C for 45 minutes in 5X SCC (0.75 M sodium citrate, 75 mM sodium citrate, pH 7.0); 0.1% SDS (sodium dodecyl sulfate) and 1% BSA (bovine serum albumin), the hybridization was carried out overnight at 42°C in buffer containing 50% formamide, 5XSSC, and 0.1% SDS. Hybridized slides were washed in stringent conditions (twice at 42°C in 1X SSC, 0.1% SDS for 2 minutes each; twice at room temperature in 0.1X SSC, 0.1% SDS for 4 minutes each; and twice at room temperature in 0.05X SSC for 2 minutes each), dried and analyzed with the GeneticMicrosystems (Affymetrix) 418 cDNA microarray scanner and associated Imagene 4.1 image analysis software according to the manufacture's recommendations.

Example 4. Identification of deregulated, disease-relevant nucleic acids.

Deregulated cDNAs according to the invention include those that were expressed by at least 2-fold higher levels in more than 50% of the 21 HCC samples subjected to the cDNA microarray expression profiling (Figure 21). These cDNAs inserts were sequenced and compared with the non-redundant and EST databases of Gen-

Bank using the BLAST algorithm (Altschul et al., 1997, Nucleic Acids Res., 25:3389-3402). The nucleic acids according to the invention were selected from the deregulated nucleic acids that fulfilled this criteria and the sequences that preferably have not previously been associated with liver disorders, especially with HCC. Expression values do not represent a normal distribution. However, evaluation of the values with nonparametric measures demonstrates significant differences in gene expression for the nucleic acids according to the invention (Figures 22-24). Moreover, comparison of data between liver disorders indicates differences in expression values in proliferative hyperplastic and malignant neoplastic liver disorder samples (Figure 24). Verification of deregulated expression in HCC of the nucleic acids according the invention was accomplished with independent methods as described in detail below.

Extensive patient information has also been assembled for each patient profiled. This information includes parameters such as age, sex, diagnosis, tumor grading scale parameters, tumor size, tissue ischemia time, alcohol use status, and follow up information (Figure 25 includes some of the diagnostic information available for patient samples employed for RT-PCR validation of deregulated nucleic acid expression in HCC).

In addition to providing a pool of candidate cDNAs highly enriched for differentially expressed genes, the SSH library represents all differentially expressed genes in HCC, including those not expressed in non-diseased liver. Consequently the SSH libraries include sequences that would not appear in conventional cDNA libraries or in genome-wide cDNA microarrays from public domain cDNA clone collections. Together these parameters better illustrate nucleic acids specifically deregulated in disorders according to the invention.

The regulation of the polypeptides and nucleic acids according to the invention is preferably essential for the pathologic process and which are thus in a direct or indirect relationship with diagnosis, prevention and/or treatment of liver disorders. The polypeptides of these genes and the nucleic acids encoding these do not belong

to the targets known until now for diagnosis, prevention and/or the treatment of liver disorders, or other cancers as indicated above, such that surprising and completely novel therapeutic approaches result from this invention.

Example 5: Independent verification of differential expression of the nucleic acids according to the invention

RNA was isolated from human patient samples as described in detail above. HCC samples for this analysis were not from the same patients as employed for production of the HCC SSH library or for cDNA microarray chip hybridization (see examples above, Figures 21 - 23). In addition to HCC samples, RNA was prepared from independent non-diseased liver samples to assess expression of the nucleic acids according to the invention in non-diseased liver tissue. Further, RNA was prepared from additional non-diseased and cancer tissues to assess expression of the nucleic acids according to the invention in other normal human tissues and other human cancers. 1 µg of RNA was converted to single-strand cDNA with the aid of Superscript reverse transcriptase (LifeTechnologies) in dATP, dCTP, dGTP, and dTTP (0.4 mM each), 7.5 nM random 6-nucleotide primer (hexamers), 10 mM dithiothreitol and 1 unit RNAse inhibitor using standard procedures known in the art (Sambrook et al., Molecular Cloning, 2nd ed., 1989, Cold Spring Harbor Press, NY, USA, pp. 5.52-5.55). The presence or absence of the nucleic acids according to the invention was then determined by amplification of these sequences from the cDNA with primer pairs specific to each nucleic acid according to the invention in PCR experiments. The primers used for this analysis are given in the following table:

Clone	SEQ ID No.	Primer 1 (SEQ ID NO.)	Primer 2 (SEQ ID NO.)
OBcl1	10	5'-CAGGTGAATTTCAAAGGAGGATTTACTCAC- 3' (22)	5'-GTGAGTAAATCCTCCTTTGAAATTCACCTG-3' (23)
OBcl5	11	5'-	5'-TGCCAGGAAACTTCTTG

		GCAAGCCAGGAAGAGT CGTCACG-3' (24)	CTTGATGC-3' (25)
IK2	12	5'-AGTAACCAGTTGAGATG AAGCACGTC-3' (26)	5'-CAGAAGAGCAACAAGA ATGGTATCCTGC-3' (27)
IK5	13	5'-AACTTGAGTTCTATTTAC CTTGCAC-3' (28)	5'-TTGCTTGGGTCATCTAA AGAC-3' (29)
DAP3	14	5'-ACTCACGTGCAAGGATG ATG-3' (30)	5'-AGCTCTCGGACTCTCAA CTG-3'(31)
LOC5	15	5'-CTTCTCCTATGACTGATC CTACTATG-3' (32)	5'-CAGGATGCAGAACTCAC CCTG-3' (33)
SEC14L 2	16	5'-GCAGATTTCCCGTGGCT CCTC-3' (34)	5'-GTTGGGCAGCACCTCTG TCATC-3' (35)
SSP29	17	5'-CTGTGACATTCCGCCTTC CTTC-3' (36)	5'-CCACGCTACTGCAAGAA TCTTAC-3' (37)
HS16	18	5'-AGAAGTTCAACCTGGAG AGATGG-3' (38)	5'-CAAGGAAGCTAGGAATG ACAGGAG-3' (39)
IK3	19	5'-GCAAAGCCAAATTCATG TTACTCT-3' (40)	5'-CAGATACGAACAGTGAA TGGAAATACG-3' (41)

These primers are also empoyable for diagnosis of disorders according to the invention, but the skilled worker may as well design other primers specific for a given nucleic acid according to the invention. The PCR included 0.5% of the cDNA, 1X Amplitaq PCR buffer, 2.5 mM $MgCl_2$, 37.5 nM each primer, 0.5 mM each of dATP, dCTP, dGTP and dTTP and 1.5 units Amplitaq DNA polymerase (Applied Biosystems). PCR conditions were optimized as needed for each primer pair, typically: 94°C for 3 minutes followed by 30 cycles of: 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 60 seconds, then cooled to 4°C. Amplification of cDNA inserts was confirmed by electrophoresis of a 5-10% of the PCR on a 1% agarose gel containing 0.5 µg/ml ethidium bromide and run in 1X Tris Acetate EDTA (TAE) buffer. Standard controls for RT-PCR including RNAse treatment of samples prior to cDNA synthesis and omission of reverse transcriptase routinely demonstrated the specificity of these reactions. Reactions were scored for expression (+) or absence of expression (-) based upon whether a discrete band of the correct molecular size was observed in the gel. Very faint or ambiguous bands under

these conditions were scored with (+/-). A summary of these verification studies in HCC and non-diseased liver is given in Figure 25. Data representative of these analyses in independent HCC and non-diseased liver samples is provided in Figure 26.

An additional independent validation of differential expression of the nucleic acids according to the invention is illustrated in Figure 27. In this case, 15 μg of RNA from two HCC samples and from non-diseased liver was subjected to denaturing electrophoretic separation on a 1% agarose gel containing 2.2 M formaldehyde and 1X MOPS buffer (10 mM 4-morpholinepropanesulfonic acid, 1 mM EDTA, 5 mM sodium acetate, pH 7.0) run in 1X MOPS buffer. The size-fractionated denatured RNA was transferred to nylon membrane (GeneScreen, New England Nuclear) with the RNA (northern) blot technique and cross linked to the membrane with UV light, all according to procedures well known to the skilled artisan (Sambrook et al., Molecular Cloning, 2nd ed., 1989, Cold Spring Harbor Press, NY, USA, pp 7.39-7.52). cDNA clone inserts of from the SSH clones for OBcl1 and OBcl5 (SEQ ID Nos. 10 and 11) were isolated by PCR amplification as described in the previous example. Single stranded radiolabeled RNA probes corresponding to these sequences were synthesized from this template using SP6 and T7 RNA polymerase in the presence of α-^{32}P-UTP in 1X labeling buffer: 0.5 mM ATP, CTP, GTP, 10 mM dithiotreitol, and 20 units of appropriate RNA polymerase at 37°C for 35 minutes. The resulting antisense probe is complementary to the corresponding mRNA sequence and thus expected to hybridize specifically to the mRNA sequence on the northern blot. Conversely, the sense probe sequence matches that of the mRNA and thereby is expected not to hybridize to the mRNA. Identical northern blots were prehybridized in 15 ml of 250 mM monobasic sodium phosphate, 250 mM dibasic sodium phosphate, 7% SDS, 1 mM EDTA and 1% BSA for at least 30 minutes at 68°C. For hybridization the prehybridization buffer was removed and replaced with 10 ml of the same buffer including the sense and each antisense RNA probes described above at 68°C overnight as described by the supplier of the buffer. The RNA blots were washed under stringent conditions (2X SSC, 0.1% SDS twice at room temperature for 15 minutes each; 1X SSC, 0.1% SDS twice at 68°C for 10 minutes each), dried and exposed to x-ray film to produce an

autoradiograph. As seen in Figure 27, each antisense probe specifically hybridizes to discrete HCC RNA but only weakly or not at all to non-diseased liver RNA. The specificity of these results is demonstrated by the absence of specific signal from the corresponding sense probe for both OBcl1 and OBcl5. In addition, RNAs of different molecular weights are apparent with the OBcl1 antisense probe. This result most likely represents discrete mRNA species, perhaps produced by alternative splicing. These species were expected based upon the finding that several different sized cDNA clones corresponding to this sequence are reported in the GenBank sequence database.

These data provide independent verification of deregulated expression of the nucleic acids according to the invention in HCC. Expression of the nucleic acids according to the invention is either absent or observed only at very low levels in non-diseased liver, thereby validating the differential expression of these nucleic acids identified by hybridization to the cDNA microarray. The results provide surprising evidence that the nucleic acids according to the invention can be used to diagnose, prevent and/or treat disorders according to the invention.

Example 6: A method of diagnosing using HCC specific probes.

A diagnostic method for disorders according to the invention preferably based on the polymerase chain reaction (PCR) can be established. A standard PCR detection of nucleic acid sequences of the invention can be sufficient to identify, for example, circulating HCC tumor cells in the blood stream of the patient. Detection of expression of nucleic acid sequences of the invention in tumor biopsy material however, such as from a fine needle biopsy, would also be a preferred indication for this diagnostic procedure. Nucleic acid sequences of the invention, OBcl5 (SEQ ID No. 11) for example, are not detected in most non-diseased tissues and relatively specifically expressed in e.g. HCC. Elevated expression of this nucleic acid in cirrhosis and HCC is also demonstrated indicating the potential discriminatory power of such an approach for differential diagnosis of liver diseases (Figure 24). The PCR diagnostic would preferably require approximately 1 pg, preferably at

least 100 ng, more preferably at least 1 µg of RNA isolated from patient material. In the preferred utilization the RNA would be isolated according to standard procedures from e.g. the white blood cell fraction preferably from circulating blood obtained by the minimally invasive venupuncture procedure. In this preferred case, the procedure would detect the presence of HCC tumor cells in the blood circulatory system. RNA could similarly be isolated from liver biopsy material. For specific detection of OBcl5, for example, the PCR diagnostic would include several primers specific for OBcl5 nucleic acid sequence, including a specific antisense primer (Primer OBcl5-p1; 5'- GCCACAGGTTGAACACTTAATTTG-3'; SEQ ID NO. 42; from nucleotide 350-327 on SEQ ID No.11) for cDNA synthesis from the RNA generated from the patient sample. Similarly specific PCR primers such as for example OBcl5-p2 (5'-AGGAAGAGTCGTCACGAGAACC-3'; SEQ ID NO. 43; from nucleotide 107-128 on SEQ ID No.11) and OBcl5-p3 (5'-ATAATGCTGTGCTTAGTTTATTGCC-3'; SEQ ID NO. 44; from nucleotide 313-289 on SEQ ID No.11). Sensitivity, specificity and quality control may be improved by the provision of an additional primer set (for example: OBcl5-p4; 5'-GATCGTGGACATTTCAACCTC-3'; SEQ ID NO. 45; from nucleotide 147-167 on SEQ ID No.11 and OBcl5-p5; 5'-TCTTGCTTGATGCTTTGGTC-3'; SEQ ID NO. 46; from nucleotide 280-261 on SEQ ID No.11) that are specific for the OBcl5 nucleic acid insert and internal (nested) to primers OBcl5-p2 and –p3.

cDNA may be prepared from the patient RNA sample following digestion of the RNA with RNAse-free DNAse-1 (Roche) to eliminate potential contamination by genomic DNA. This contamination possibility is further controlled by including primers for PCR amplification from sequences of different exons of the OBcl5 gene such that PCR products resulting from a genomic DNA template (and thereby not reflective of expression of the mRNA corresponding to OBcl5) would be larger than the RNA specific PCR products. cDNA synthesis can e.g. be primed by the OBcl5 specific OBcl5-p1 (at about 1 µM) with the aid of reverse transcriptase [such as Maloney murine leukemia virus reverse transcriptase (Roche) at about 2 unit/reaction] in an appropriate buffer such as 50 mM Tris-HCl, 6 mM MgCl2, 40 mM KCl, and 10 mM dithiotreitol, pH 8.5. Also required in the cDNA synthesis reaction is dATP, dCTP, dGTP and dTTP, each at about 1 mM, RNAse inhibitor,

such as placental RNAse inhibitor (Roche) at about 1-10 units/reaction. cDNA synthesis would be preferably carried out at 42°C for 30 to 60 minutes followed by heating at 95°C for 10 minutes to denature the RNA template. The resulting cDNA can be employed as the template for a PCR to detect OBcl5 in the blood (or liver biopsy sample). The additional reagents required for PCR detection of OBcl5 would preferably also be provided including: 10X Taq DNA polymerase buffer (500 mM Tris-Cl pH 8.3, 25 mM $MgCl_2$, 0.1% Triton X-100); a mixture of dATP, dCTP, dGTP and dTTP for a final concentration of 0.2 mM each; Taq DNA polymerase (2.5U/reaction), and OBcl5 specific primers such as OBcl5-p2, OBcl5-p3, OBcl5-p4, and OBcl5-p5 (0.1 - 1 μM final concentration). A positive control for PCR amplification such DNA from a plasmid clone with the OBcl5 sequence insert would preferably also be included (1-10 ng/reaction). The PCR can e.g. be carried out over 22-40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds. As indicated above, preferred additional sensitivity and specificity may be achieved in this diagnostic procedure by utilization of the additional OBcl5 primer set located within the sequence amplified with the original PCR primer set. In this case a subsequent PCR under conditions similar to those utilized in the first PCR reaction except that preferably primers OBcl5-p4 and OBcl5-p5 would be employed to amplify the nested sequence in a reaction that included 1-10 μl of the first PCR as the template DNA. Alternatively, the reaction may preferably be carried with the first primer set (OBcl5-p2 and OBcl5-p3) for 10-15 cycles after which and 1-10 μl of this reaction then included as template in a new PCR reaction with primers OBcl5-p4 and OBcl5-p5 (and including all the necessary PCR components). Detection of OBcl5 specific PCR product(s) should preferably utilize agarose gel electrophoresis as is known in the art and described in previous examples. Included in the diagnostic should preferably be a comparable fluid or tissue extract as a control for such PCR-based diagnostic test. This may include serum or plasma from non-diseased individuals and/or serum, plasma or tissue extracts from an appropriate animal model. If the PCR-determined expression of the nucleic acid according to the invention such as the product of the reaction with primers OBcl5-p4 and OBcl5-p5 is upregulated in the sample isolated from the patient relative to the control and if in particular the upregulated expression essentially matches the disorder specific (mean) expression ratios such as those illustrated in figure 21 then such matching is indicative of the patient suffering from the disorder.

Variations on this approach can also be appreciated. The cDNA synthesis and PCR amplifications can be carried our sequentially or simultaneously in a single reaction vessel utilizing heat stabile DNA polymerases with reverse transcriptase activities, such as provided by the Titan one-tube or *Carboxydothermus* DNA polymerase one-set RT-PCR systems from Roche. Alternatively the PCR product can be monitored by incorporation of fluorescently-labeled primers or various fluorescence-based indicators of PCR product including the Taqman probe hydrolysis systems and fluorescent double-stranded DNA intercalating molecules such as SYBR green. The fluorescent-based approaches provide advantage as the accumulation of PCR product can be continuously monitored to achieve sensitive quantitative assessment of expression of the nucleic acid according to the invention. This should be particularly advantageous for nucleic acids increased in blood or tissues of disorders according to the invention but also present at lower levels in non-diseased patients and tissues such that quantitative information about the level of expression of the nucleic acid is acquired. Further, as with this example, accurate quantitation of nucleic acid expression levels contributes to differential diagnosis, between cirrhosis and HCC for example. Comparison of this data with supplied standards indicative of disease and absense of disease provides an important advantage for such a diagnostic procedure.

Additional variations on this diagnostic strategy include simultaneous detection of multiple nucleic acids according to the invention and/or of nucleic acids according to the invention together with other nucleic acids implicated in the disorder. Further hybridization-based diagnostic detection of nucleic acids according to the invention is also envisioned. In this case mRNA detection preferably utilizing RNA blot, RNAse protection or in situ hybridization on patient cells or tissue biopsy samples is also effective.

By similar methods and variants thereof the nucleic acids according to the invention and/or of nucleic acids according to the invention together with other nucleic acids can be utilized for diagnosis of the disorders according to the invention.

Example 7: A method of diagnosing via antibody detection of polypeptides according to the invention

A preferred diagnostic method for disorders according to the invention is based on antibodies directed against a polypeptide according to the invention. For example, a diagnostic procedure may preferably employ serum detection of specific upregulated gene proteins via enzyme-linked immunosorbent assay (ELISA) assay. In a simple form the diagnostic assay preferably includes an microtiter plate or strip of microtiter wells, e.g., thoroughly coated with an isolated and purified antibody specific to a polypeptide according to the invention such as OBcl5.pr (SEQ ID No. 2). The antibody may for example be an affinity purified polyclonal antibody, such as is commonly raised in rabbits, for example, or a purified monoclonal antibody such as is commonly produced in mice according to procedures well established in the art (Cooper, H.M. & Paterson, Y., (2000), *In* Current Protocols in Molecular Biology (Ansubel, F.A. et al., eds.) pp. 11.12.1 – 11.12.9, Greene Publ. & Wiley Intersci., NY); (Fuller S.A. et al., (1992), *In* Current Protocols in Molecular Biology (Ansubel, F.A. et al., eds.) pp. 11.4.1 – 11.9.3, Greene Publ. & Wiley Intersci., NY). Preferably, the antibody may a recombinant antibody obtained from phage display library panning and purification as has been described by Knappik et al. (2000, J. Molec. Biol. 296:57-86) or by Chadd and Chamow (2001 Curr. Opin. Biotechnol. 12:188-94) or a fragment thereof. The antibody coating is preferably achieved by dilution of the anti-OBcl5.pr antibody to 1-100 μg/ml in a standard coating solution such as phosphate buffered saline (PBS). The antibody is preferably bound to the absorptive surface of the microtiter well (such as a Nunc Maxisorp immunoplate) for 60 minutes at 37°C, or overnight at room temperature or 4°C. Prior to binding sample to the coated wells, the wells are preferably thoroughly blocked from non-specific binding by incubation for 15-60 minutes at room temperature in a concentrated protein solution such as 5% bovine serum albumin in phosphate buffered saline or 5% non-fat dry milk powder resuspended in the same buffer. Preferably, the patient sample material is then applied to the microtiter wells, diluted into the blocking solution to increase specificity of detection. The sample may be for example plasma or serum or protein extract from tissue biopsy

or surgical resection prepared according to methods well known in the art (Smith, J.A. (2001) *In*, Current Protocols in Molecular Biology, Ausubel, F.A. et al., eds) pp. 10.0.1- 10.0.23, Greene Publ. & Wiley Intersci., NY). In particular, the patient sample is brought into contact with the antibody-coated well for 30-120 minutes (or longer) at room temperature or at 4°C. Non-specifically interacting proteins are preferably removed by extensive washing with a standard wash buffer such as 0.1 M Tris-buffered saline with 0.02-0.1% Tween 20, for example. Washes are preferably carried out for 3-10 minutes and repeated 3-5 times. Detection of OBcl5.pr polypeptide in the patient sample is for example achieved by subsequent binding reaction with a second, independent anti-OBcl5.pr antibody, generated as described above, recognizing a distinct epitope on the OBcl5.pr polypeptide in the standard two-site 'sandwich' type ELISA. Binding of the second anti-OBcl5.pr antibody is for example achieved by incubating the wells in the antibody (at a concentration of 1-100 μg/ml in blocking solution, for example) at room temperature for 30-60 minutes followed by extensive washing as in the previous step. The second antibody may preferably be directly coupled to an enzyme capable of producing a colorigenic or fluorgenic reaction product in the presence of an appropriate substrate, such as alkaline phosphatase. Alternatively, for example an anti-species and anti-isotype specific third antibody, so coupled to an enzyme, is employed to generate a reaction product that preferably can be detected in a standard spectrophotometric plate reader instrument. For the reaction product development, the washed (as above) antibody-antigen-enzyme complex is preferably exposed to the colorigenic substrate, such as AttoPhos from Roche for about 10 minutes at room temperature, the reaction may be stopped with a low pH buffer such as 50 mM Tris-HCl pH 5.5, or can instead be directly assayed. The amount of specifically bound OBcl5.pr polypeptide is for example determined by measurement of the amount of the enzymatic reaction product in each well following excitation at the appropriate wavelength in the spectrophotometer (420 nm in this case). Measurement is preferably made in the plate reader at the emission wavelength (560 nm in this case). Preferably included in the diagnostic is an OBcl5 protein standard, such as purified recombinant OBcl5.pr polypeptide, for example. A dilution series of this protein standard is preferably included in parallel in the ELISA as a control for the reactions and to deduce a protein standard curve for comparison of polypeptide expression levels as is well known in the art. A concentration range corresponding indicative of the

particular liver disorder(s) should preferably be provided in the diagnostic. In addition, a comparable fluid or tissue extract should preferably also be included as a control for such ELISA test. This may preferably include serum or plasma from non-diseased individuals and/or serum, plasma or tissue extracts from an appropriate animal model. Such ELISA detection diagnostics are common in the art (see for example, Hauschild et al., 2001, Cancer Res. 158:169-77). The sample:control protein levels determined by ELISA are compared with ELISA-determined disorder specific protein expression ratio values preferably determined in pathologist-confirmed tissues of patients suffering from a disorder according to the invention in relation to control samples. In case the protein level of the sample:control essentially matches the disorder specific protein expression ratio values such matching is preferably indicative of the patient suffering from the disorder. Preferably such diagnosis is carried out for more than 1 polypeptide according to the invention.

In addition the diagnostic may be directed to detecting an endogenous antibody directed against a polypeptide according to the invention or a functional variant thereof or fragment thereof present in the sample isolated from a patient which antibody or fragment thereof is directed against a polypeptide according to the invention. Detection of such autoimmune antibodies may be accomplished by methods generally known to the skilled artisan, e.g. by immunoaffinity assays such the ELISA described in detail above using polypeptides according to the invention or functional variants thereof or parts thereof as a probe. The presence of such autoimmune antibodies is indicative of the patient suffering from a disorder according to the invention.

In addition or alternatively, a relevant diagnostic kit based upon immunohistochemical detection of at least one polypeptide according to the invention can be formulated. In such a kit, for example a purified antibody or antibodies specific for the polypeptide(s) according to the invention can be included as well as preferably the reagents necessary to detect the binding of the antibody(ies) to patient cells or tissue sections. These reagents include, for example a specific anti-species and subtype specific secondary antibody -directed against a polypeptide according to the invention of a functional variant thereof- preferably coupled to an enzyme capable

of catalysis of e.g. a colorigenic substrate or coupled to a fluorophore (such as Texas Red, for example). Perferably the enzymatic substrate would also be included as well as washing and incubation buffers. An additional optional component of such a kit may be a section of positive control tissue, e.g. liver, or tissues or a section from a packed pellet of cells specifically expressing the polypeptide(s) as a positive tissue control. Instructions provided would include preferred and/or alternative methods of antigen retrieval for detection of the polypeptide(s) according to the invention or e.g., indication that frozen, rather than formalin fixed and paraffin-embedded tissue material should be employed. In this case, recommendations would preferably be included for fixation of frozen tissue sample sections, such as immersion in ice-cold acetone for 10 minutes. Further instructions would preferably provide recommendations for the concentration of antibodies to use in the detection of the gene product(s) as well as e.g., recommended and suggested incubation times and temperatures for exposure of the tissue to the immunological reagents provided. Preferred reaction buffers for the antibody incubations, such as 0.01% - 0.1% tween-20 containing phosphate buffered saline including 3% normal sheep serum, could also be included. Further, specific conditions for washing of the tissue sections prior to and following incubation in the specific antibody would be preferably included, such as for example, 4 washes with 0.1% tween-20 containing phosphate buffered saline for 5 minutes each. Such immunohistochemical detection protocols are known to a person skilled in the art. In general the kit would preferably include a panel of images of specific immunohistochemical staining results from positive and negative tissue examples and in particular tables indicating which result is indicative of the patient suffering from the discorder to be diagnosed as a user guide. Utilization of such a kit would preferably rule out, support or confirm diagnoses of the aforementioned liver disorders, liver cancer, or epithelial cancers according to the invention.

As specified above for nucleic acid-based diagnostic approaches, diagnostics based on detection and/or quantitation of polypeptides according to the invention may include 1 or more of such polypeptides. Moreover, simultaneous detection of such polypeptides together with other peptides implicated in the disorders according to the invention may be employed in such diagnostics.

September 27, 2002
O37456EP BÖ/HvC

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Patent Claims

1. Diagnostic containing the nucleic acids according to SEQ ID No. 10 to SEQ ID No. 19 or variants thereof, combined with suitable additives and/or auxiliaries.

2. A diagnostic according to claim 1, wherein the nucleic acid is a probe, preferentially a DNA probe.

3. The use of the nucleic acids according to SEQ ID No. 10 to SEQ ID No. 19 or variants thereof for the production of a diagnostic for the diagnosis of a liver disorder and an epithelial cancer, wherein the nucleic acids according to SEQ ID No. 10 to SEQ ID No. 19 or variants thereof are combined with suitable additives and/or auxiliaries.

4. The use of according to claim 3, wherein the liver disorder is a disorder selected from cirrhosis, alcoholic liver disease, chronic hepatitis, Wilson's Disease, heamochromatosis, hepatocellular carcinoma, benign liver neoplasms, and focal nodular hyperplasia.

5. The use according to claim 3, wherein the epithelial cancer is an adenocarcinoma of any organ other than liver, preferably of an organ selected from the group consisting of the lung, the stomach, the kidney, the colon, the prostate, the skin, and the breast.

September 27, 2002
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Abstract

The invention relates to polypeptides, nucleic acids encoding these or a nucleic acid according to SEQ ID No. 19 and to their use for the diagnosis, prevention and/or treatment of liver disorders and neoplastic disorders especially liver cancer and/or other epithelial cancers. In particular, the present invention relates to the use of these nucleic acids and polypeptides in connection with hepatocellular carcinoma (HCC), other epithelia-derived cancers, and non-malignant and non-cancerous liver disorders (e.g., adenoma, focal nodular hyperplasia, cirrhosis).

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Figure 1:

Molecule	Polypeptide (SEQ ID No.)	Accession number	DNA (SEQ ID No.)	Accession number
OBcl1	1	NP_443111	10	AL833272
OBcl5	2	Novel	11	Novel
IK2	3	NP_079436	12	NM_025160
IK5	4	NP_006398	13	NM_006407
DAP3	5	NP_387506	14	NM_033657
LOC5	6	NP_060917	15	NM_018447
SEC14L2	7	NP_036561	16	NM_012429
SSP29	8	NP_006392	17	NM_006401
HS16	9	*NP_057223*	18	NM_016139
IK3		--	19	AL049338

Figure 2: OBcl1.pr (SEQ ID No. 1)

```
1   MYSSSCETTRNTTGIEESTDGMILGPEDLS
31  YQIYDVSGESNSAVSTEDLKECLKKQLEFC
61  FSRENLSKDLYLISQMDSDQFIPIWTVANM
91  EEIKKLTTDPDLILEVLRSSPMVQVDEKGE
121 KVRPSHKRCIVILREIPETTPIEEVKGLFK
151 SENCPKVISCEFAHNSNWYITFQSDTDAQQ
181 AFKYLREEVKTFQGKPIMARIKAINTFFAK
211 NGYRLMDSSIYSHPIQTQAQYASPVFMQPV
241 YNPHQQYSVYSIVPQSWSPNPTPYFETPLA
271 PFPNGSFVNGFNSPGSYKTNAAAMNMGRPF
301 QKNRVKPQFRSSGGSEHSTEGSVSLGDGQL
331 NRYSSRNFPAERHNPTVTGHQEQTYLQKET
361 STLQVEQNGDYGRGRRTLFRGRRRREDDRI
391 SRPHPSTAESKAPTPKFDLLASNFPPLPGS
421 SSRMPGELVLENRMSDVVKGVYKEKDNEEL
451 TISCPVPADEQTECTSAQQLNMSTSSPCAA
481 ELTALSTTQQEKDLIEDSSVQKDGLNQTTI
511 PVSPPSTTKPSRASTASPCNNNINAATAVA
541 LQEPRKLSYAEVCQKPPKEPSSVLVQPLRE
571 LRSNVVSPTKNEDNGAPENSVEKPHEKPEA
601 RASKDYSGFRGNIIPRGAAGKIREQRRQFS
631 HRAIPQGVTRRNGKEQYVPPRSPK
```

Figure 3: OBcl5.pr sequence (SEQ ID No. 2)

1 MGVELMMELE PLQGNEETRA LFMPREDTAR PQSASQEESS
41 REPDHAGTLI VDISTSRTVI QNAYVSLEET LK

Figure 4: IK2.pr sequence (SEQ ID No.3)

1 MLPPRRLQTL LRQAVELQRD RCLYHNTKLD NNLDSVSLLI
41 DHVCSRRQFP CYTQQILTEH CNEVWFCKFS NDGTKLATGS
81 KDTTVIIWQV DPDTHLLKLL KTLEGHAYGV SYIAWSPDDN
121 YLVACGPDDC SELWLWNVQT GELRTKMSQS HEDSLTSVAW
161 NPDGKRFVTG GQRGQFYQCD LDGNLLDSWE GVRVQCLWCL
201 SDGKTVLASD THQRIRGYNF EDLTDRNIVQ EDHPIMSFTI
241 SKNGRLALLN VATQGVHLWD LQDRVLVRKY QGVTQGFYTI
281 HSCFGGHNED FIASGSEDHK VYIWHKRSEL PIAELTGHTR
321 TVNCVSWNPQ IPSMMASASD DGTVRIWGPA PFIDHQNIEE
361 ECSSMDS

Figure 5: IK5.pr sequence (SEQ ID No.4)

1 MDVNIAPLRA WDDFFPGSDR FARPDFRDIS KWNNRVVSNL
41 LYYQTNYLVV AAMMISIVGF LSPFNMILGG IVVVLVFTGF
81 VWAAHNKDVL RRMKKRYPTT FVMVVMLASY FLISMFGGVM
121 VFVFGITFPL LLMFIHASLR LRNLKNKLEN KMEGIGLKRT
161 PMGIVLDALE QQEEGINRLT DYISKVKE

Figure 6: DAP3.pr sequence (SEQ ID No.5)

1 MMLKGITRLI SRIHKLDPGR FLHMGTQARQ SIAAHLDNQV
41 PVESPRAISR TNENDPAKHG DQHEGQHYNI SPQDLETVFP
81 HGLPPRFVMQ VKTFSEACLM VRKPALELLH YLKNTSFAYP
121 AIRYLLYGEK GTGKTLSLCH VIHFCAKQDW LILHIPDAHL
161 WVKNCRDLLQ SSYNKQRFDQ PLEASTWLKN FKTTNERFLN
201 QIKVQEKYVW NKRESTEKGS PLGEVVEQGI TRVRNATDAV
241 GIVLKELKRQ SSLGMFHLLV AVDGINALWG RTTLKREDKS
281 PIAPEELALV HNLRKMMKND WHGGAIVSAL SQTGSLFKPR
321 KAYLPQELLG KEGFDALDPF IPILVSNYNP KEFESCIQYY
361 LENNWLQHEK APTEEGKKEL LFLSNANPSL LERHCAYL

Figure 7: LOC5.pr sequence (SEQ ID No.6)

1 MAGPELLLDS NIRLWVVLPI VIITFFVGMI RHYVSILLQS
41 DKKLTQEQVS DSQVLIRSRV LRENGKYIPK QSFLTRKYYF
81 NNPEDGFFKK TKRKVVPPSP MTDPTMLTDM MKGNVTNVLP
121 MILIGGWINM TFSGFVTTKV PFPLTLRFKP MLQQGIELLT
161 LDASWVSSAS WYFLNVFGLR SIYSLILGQD NAADQSRMMQ
201 EQMTGAAMAM PADTNKAFKT EWEALELTDH QWALDDVEEE
241 LMAKDLHFEG MFKKELQTSI F

Figure 8: SEC14L2.pr sequence (SEQ ID No.7)

1 MSGRVGDLSP RQKEALAKFR ENVQDVLPAL PNPDDYFLLR
41 WLRARSFDLQ KSEAMLRKHV EFRKQKDIDN IISWQPPEVI
81 QQYLSGGMCG YDLDGCPVWY DIIGPLDAKG LLFSASKQDL
121 LRTKMRECEL LLQECAHQTT KLGRKVETIT IIYDCEGLGL
161 KHLWKPAVEA YGEFLCMFEE NYPETLKRLF VVKAPKLFPV
201 AYNLIKPFLS EDTRKKIMVL GANWKEVLLK HISPDQVPVE
241 YGGTMTDPDG NPKCKSKINY GGDIPRKYYV RDQVKQQYEH
281 SVQISRGSSH QVEYEILFPG CVLRWQFMSD GADVGFGIFL
321 KTKMGERQRA GEMTEVLPNQ RYNSHLVPED GTLTCSDPGI
361 YVLRFDNTYS FIHAKKVNFT VEVLLPDKAS EEKMKQLGAG
401 TPK

Figure 9: SSP29.pr sequence (SEQ ID No.8)

1 MDMKRRIHLE LRNRTPAAVR ELVLDNCKSN DGKIEGLTAE
41 FVNLEFLSLI NVGLISVSNL PKLPKLKKLE LSENRIFGGL
81 DMLAEKLPNL THLNLSGNKL KDISTLEPLK KLECLKSLDL
121 FNCEVTNLND YRESVFKLLP QLTYLDGYDR EDQEAPDSDA
161 EVDGVDEEEE DEEGEDEEDE DDEDGEEEEF DEEDDEDEDV
201 EGDEDDDEVS EEEEEFGLDE EDEDEDEDEE EEEGGKGEKR
241 KRETDDEGED D

Figure 10: HS16.pr sequence (SEQ ID No.9)

1 MPRGSRSRTS RMAPPASRAP QMRAAPRPAP VAQPPAAAPP
41 SAVGSSAAAP RQPGLMAQMA TTAAGVAVGS AVGHTLGHAI
81 TGGFSGGSNA EPARPDITYQ EPQGTQPAQQ QQPCLYEIKQ
121 FLECAQNQGD IKLCEGFNEV LKQCRLANGL A

Figure 11: OBcl1 sequence (SEQ ID No.10)

1 ccgggtggag gggcaaggcg agtgtgtgtc cttatcctag caattggggc gcgggcctgt
61 gagccagttg gagttgcggc ggcgggaacg attgggctga gcagaggacg acatgttgct
121 tttcgtggag catttatggg gttaagtggc atgggatttc tgtttctgat agtaaatagc
181 aggtagcatc taaaggaact ggtttaaatc ctaatgccaa agtatggcaa gaaattgctc
241 ctggaaatac tgatgccacc ccagtaactc atggaactga aagctcttgg catgaaatag
301 cagctacatc aggtgctcat cctgagggta atgcagagct ctcagaagat atatgtaaag
361 aatatgaagt aatgtattct tcatcttgtg aaaccacaag aaatactaca ggcattgaag
421 aatcaactga tgggatgatt ttaggaccag aagatctgag ttaccaaata tatgatgttt
481 ccggagaaag caattcagca gtttctacag aagacctaaa agaatgtctg aagaaacaat
541 tagaattctg ttttttcacga gaaaatttgt caaaggatct ttacttgata tctcaaatgg
601 atagtgatca gttcatccca atttggacag ttgccaacat ggaagaaata aaaaagttga
661 ctacagaccc tgatctaatt cttgaagtgt taagatcttc tcccatggta caagttgatg
721 agaagggtga gaaagtgaga ccaagtcata agcgttgtat tgtaattctt agagagattc
781 ctgaaacaac accaatagag gaagtgaaag gtttgttcaa aagtgaaaac tgccccaaag
841 tgataagctg tgagtttgca cacaatagca actggtatat cactttccag tcagacacag
901 atgcacaaca ggcttttaaa tacttaagag aagaagttaa aacatttcag ggcaagccaa
961 ttatggcaag gataaaagcc atcaatacat tttttgctaa gaatggttat cgattaatgg
1021 attctagtat ctatagtcac cccattcaaa ctcaagcaca gtatgcctcc ccagtcttta
1081 tgcagcctgt atataatcct caccaacagt actcggtcta tagtattgtg cctcagtctt
1141 ggtctccaaa tcctacacct tactttgaaa caccactggc tccctttccc aatggtagtt
1201 ttgtgaatgg ctttaattcg ccaggatctt ataaaacaaa tgctgctgct atgaatatgg
1261 gtcgaccatt ccaaaaaaat cgtgtgaagc ctcagtttag gtcatctggt ggttcagaac
1321 actcaacaga gggctctgta tccttggggg atggacagtt gaacagatat agttcaagaa
1381 actttccagc tgaacggcat aaccccacag taactgggca tcaggagcaa acttaccttc
1441 agaaggagac ttccactttg caggtggaac agaatgggga ctatggtagg ggcaggagaa
1501 ctctcttcag aggtcgaaga cgacgagaag atgacaggat ctcaagacct catccttcaa
1561 cagctgaatc aaaggctcca acaccaaagt ttgacttatt agcctcaaat tttccacctt
1621 tacctggaag ttcatcaaga atgccaggtg aactcgtttt ggagaatagg atgtctgatg
1681 ttgttaaagg tgtctacaaa gaaaaggata atgaagagtt gacaattagt tgcccagtgc
1741 ctgcagatga gcagacagaa tgcacttctg cccagcaact caatatgagt accagttctc
1801 catgtgctgc tgagcttact gcattaagca caactcagca agaaaaggat ctaatagaag
1861 attcctctgt tcagaaggat ggtctcaatc agacaactat accagtttct cctccaagta

1921 ctacaaagcc atcgagggca agtactgctt caccatgtaa taataacata aatgcagcta
1981 cagctgtggc tctacaggaa ccccgaaagt taagttatgc tgaagtgtgc cagaagcccc
2041 ctaaagagcc atcttcagtt cttgtgcagc cactacggga acttcgctcc aatgtggtgt
2101 ctcccaccaa aaatgaagac aatggagctc ctgagaactc cgttgagaaa ccacatgaga
2161 agccagaagc aagggctagt aaggattatt ctggcttccg aggcaatata atccccaggg
2221 gagcagcagg aaaaatcagg gaacagagac gccagtttag ccatagggct atacctcagg
2281 gagtgactcg acgtaatggc aaagagcaat atgtgccacc cagatcacca aagtaaaaaa
2341 caacaaaact attcaaaaac ttcactctct tcccattaaa cttgaactgt ggctatattg
2401 aactgttttg gaggggaggg ggtagccagg aaggaaacaa gagaaagtac gtccatttca
2461 ttatggattt tggagttgtg agtgatagga tcccaaaatt catctctaat gtggttttta
2521 aatgctggag gattccaatc aatataaata tatatatata tatacacaca catatataaa
2581 aagtataatt tttctatttt tgtttttggt tttaatttgc agagatttgc tgccaggaat
2641 caattttgag ggttcagatt tagcttggaa gaaaaaaaag aaacatacat ccttcagtat
2701 aggagatgag ggaatgagag aaaatatttt ttgaagaagc atttctgtaa aattagaaat
2761 tacttttttt aatctattta aagtttggct tgaagaatgc catctctgac tatatggcct
2821 tgtattgcaa agcagatcag tggctggggt gcctgttgtg ggtgtgagtg tgtacaagag
2881 cgattgaagc caaatctgtt gtcatgttag taaatgattt gaaaactgaa tgtaatactt
2941 gagtagattt ttttttctag tttgaaattt agtctgtctt tttgacctta ctaatatttc
3001 atttaacaag ttgtaaaact ctgattgtac ttagagatgt gactaccaat cagtttgata
3061 ctcaaggaaa gggggttatt caagaaattg aaaatttcat cttggacctc agtgcatcgg
3121 tcaaatggat ttcagaggtt taaacttccc tgtgattccc cctgaatacc cccaaaatga
3181 gaaacaaaat ttttttctt actccatttg ttactctctg ttctttgact gcccacccac
3241 agaaaagcaa aataaccaac tacctactca attgtgtgtt tgtaattgct ttgagcagtc
3301 tagtcaaatc atataaattg ttctaaattt cagaattgaa cattgaagta ttaactcttc
3361 tgttcacaca tttagaattt tagctcccaa gatggtaggg cagactgacc gtacagtaat
3421 ttatttgtcg ttagtgttaa agattaagca tagtaactga ctcttaagtg ttaaataatg
3481 tagaagtaaa aaaatttttt ttaaaggctt aatttgggag gggggactta tttctgttta
3541 cagtgtatta ccttccttcc ctcctcttct ccccccacac ccaacaaaat acagtttgga
3601 attcactgaa acagtaccag caagtcatga gatttttag taaagatgag aaagatggtt
3661 gaagaaaatt agtgcataat ttctcagtga ataaagttgt agctctcata tactaaatag
3721 acaagtttac atgctgttat ttagaaaatg actaaaatat taaaaaccgt gttgtgttaa
3781 tctgttttaa gtcataccat gttcagagtt ctatgtaagg tgggttttat ttttctttta
3841 agggatagtt tgtaatagta agaactgtcc catatgttag taaattacat atgtacaaat
3901 tgaaactgta aattgtgaac actggaaagc accattgtga catagagtaa acatcttagt

3961 aatatattaa agtgaatgta aatggtggtt aaaattacat tactgtgaaa ttcatcttcc
4021 aactctaagt taagctttgg agatacatgt tagtggttaa ctgttaagag ctttgaaaac
4081 actgcacata tctgtacaag ccagaattac tatttctttg acttattatt agcttggcag
4141 ttgcttttga tttgattgtt ttatgacatg gtatactact atatttactc agtttgaaac
4201 tattcatttc tacacactat ttttaaaaat tgcctactag gtgaaacata acaataaaac
4261 tacctgtgct gaaatttggg ggaagtttag gtcctttaaa aaaacatatt aatcattgac
4321 tacatctatg ataaaagtgc ttatttggt ttactaagat aatgcagttg gtggaaatga
4381 taaacgtttt aagtgttaac atcctttgaa tgcgttggat ttcagagaat aaacattttg
4441 taaaaatcac ttggtaagga ttataaactt aattactgca cttaaaatga aacattactt
4501 tttttaaaca atgtgtcaca aatgtaggtc tgtattactt gtatgcttgt gtgacttact
4561 gttagtccag ctctaaaaat ttaaaggttg taattgaaat acaagaaaag agccttcttt
4621 tagaagaaag caagtatatt tttgctttta cttcaaatgt tatttaaagt agaaatttaa
4681 tttgtagata taacctttaa aaattttctc attaagacaa tgtttttaat ttaatttgcc
4741 tcattacatc taatagttcc catttgatgg catgtatagg gaagagtgag agagtgtgtg
4801 tgtgtgtatg tgtgtgtaat atttatatat attcacagta tgtatttagc atttatttta
4861 ttacagcaga tttaaagttt gtatctaaat aatgcctatg agttgtgtga agctcttggc
4921 ttttttcca acgttacttt gtaactaatg agggtggatg ttcattgtag tttatttatt
4981 tggttcttta gatggaggaa tttaaaaaat caaatttttc tcttcacctt tatgacttga
5041 catttccttg atctgttgga ggctaaaagt aggtataaat gatattgaat gttgggtata
5101 gtgatactct gccatagttc ttactgcatg aagagaacaa gagtcacaca agttcaccac
5161 tttgcacttc atagagaagg tacatagaga cattgcaaaa cctgtctcca tttgctatcc
5221 tgataattaa ggttttcata atacctaggg cctgtctctg agtaatttta attttgccaa
5281 atacactgac atttaaaata gtgatccatc taaatttttt tcagctgggt tttgaggaat
5341 ataagagctt tcaatgataa aggtttgttg tagttgtctt atgtgctgaa tttgcagatg
5401 atcagatgct gtgcagaatt ctgatttatt tttgtttcct aaaattaaga tagcttgaat
5461 attatttcac attccttttt ctttttaaa taaacaggtt tgctttggaa aggcttaatg
5521 atggaatgtt agcatcttca ctagggtaaa gaagaacaaa aagaatgttg ctggaacgta
5581 aaatagtatt taaaagttaa tgaacacttc tctagtttc ttagttatgg ccttaataat
5641 tagtctcttg gcttaaatgt ccactggttt tactttgaca cagttgaaca acactggggt
5701 taagtctctg gtatttaggc tggcaatata tatattaacc atattttaaa agtaccaatt
5761 ttgtttttac agaaaagata aaactcaaaa gagaacagtg tattccttct gaggggcttt
5821 tataaattat taactataat atatgatgga ttttttccta attttttata tttccttaca
5881 attttggtgg ccattaattt aactttaggc ttttgggcat atgctagtct gagcttccga
5941 aaagatacat atatgtttcc cttttcatta gctgaatgag gatattttaa gaagttgaaa

6001 gagaatttat tttcaagttg tgagtaaatc ctcctttgaa attcacctga ttattagata
6061 acttaaagtt tatttttaaa agctgacaac tttttatgaa tcttcgagtt gacagttcct
6121 aaaagcgtaa ctcagatatt aatgggctgt gtattaaatg gttttatttt cagttttgca
6181 gcacagaaca ctgttgaaat atccatatca acttgatttt tttaacctaa ttcaggtgtc
6241 ctttgcatct cttaaatgtt gggggtgggg gtcagagcca gttatccggc ttctgttttg
6301 tcgattgctt agatttgttc ctgttgtcaa aactgttacc cccaaaattg gtgtgacaca
6361 tgctcatgca taaaatgtta aaatgagtac atccttgtat ttgtatttgt tttcaacatc
6421 gccaaggtgc tatgggaaat taacaaaatt agaaaaaaaa taaaattatt aaaaagcaaa
6481 aaaaaaaaaa aaaaaaa

Figure 12: OBcl5 sequence (SEQ ID No.11)

1 atgggggtgg aactcatgat ggaattggag cctttacaag ggaatgaaga gacaagagct
61 ctctttatgc cacgtgagga tacagcaagg ccccaatctg caagccagga agagtcgtca
121 cgagaaccag accatgcagg aactctgatc gtggacattt caacctccag aactgtgatc
181 caaaatgcat atgtatcttt ggaagaaact ctgaagtaaa ggccggaata ttctttgttt
241 aaaacattaa aaacaaaaca gaccaaagca tcaagcaaga agtttcctgg caataaacta
301 agcacagcat tatttttaa ggaacacaaa ttaagtgttc aacctgtggc aaatttgtac
361 tttctccctg aattatgttg ttatcaaaga aaaaaattgg gaagcatggc aaaatatcat
421 caaaactgaa actagaatta aactaaatta aaataaaaaa aaaaaaaaaa aaaaaaaaaa
481 aaaa

Figure 13: IK2 sequence (SEQ ID No.12)

1 ctacgtgcaa aagcagaatg ggaaggctaa gggacagctt cccgatctaa actattggat
61 aaacttcaga cctatttacc accatcagtg atgcttcccc cacggcgttt acagactctc
121 ctgcggcagg cggtggaact acaaagggat cggtgcctat atcacaatac caaacttgat
181 aataatctag attctgtgtc tctgcttata gaccatgttt gtagtaggag gcagttccca
241 tgttatacgc agcagatact tacggagcat tgtaatgaag tgtggttctg taaattctct
301 aatgatggca ctaaactagc aacaggatca aaagatacaa cagttatcat atggcaagtt
361 gatccggata cacacctgct aaaactgctt aaaacattag aaggacatgc ttatggcgtt
421 tcttatattg catggagtcc agatgacaac tatcttgttg cttgtggccc agatgactgc
481 tctgagcttt ggctttggaa tgtacaaaca ggagaactaa ggacaaaaat gagccagtct
541 catgaagaca gtttgacaag tgtggcttgg aatccagatg ggaagcgctt tgtgactgga
601 ggtcagcgtg ggcagttcta tcagtgtgac ttagatggta atctccttga ctcctgggaa
661 ggggtaagag tgcaatgcct ttggtgcttg agtgatggaa agactgttct ggcatcagat
721 acacaccagc gaattcgggg ctataacttc gaggacctta cagataggaa catagtacaa
781 gaagatcatc ctattatgtc ttttactatt tcaaaaaatg gccgattagc tttgttaaat
841 gtagcaactc agggagttca tttatgggac ttgcaagaca gagttttagt aagaaagtat
901 caaggtgtta cacaagggtt ttatacaatt cattcatgtt ttggaggcca taatgaagac
961 ttcatcgcta gtggcagtga agatcacaag gtttacatct ggcacaaacg tagtgaactg
1021 ccaattgcgg agctgacagg gcacacacgt acagtaaact gtgtgagctg gaacccacag
1081 attccatcca tgatggccag cgcctcagat gatggcactg ttagaatatg gggaccagca
1141 ccttttatag accaccagaa tattgaagag gaatgcagta gcatggatag ttgatggtga
1201 atttggagca gacgacctct gtttaactta aaattagtcg tattttaatg gcttgggatt
1261 tggtgcaaac aaacatgatt gatagctgga cagacatgct cgtcatgaaa aaagaaccat
1321 ttctgaagcc cgattggggc caaacattta caccttgctt catagtaacc agttgagatg
1381 aagcacgtcg ttagaacgtt gttggacacc atgttgaatt attcccccat cggttgtgaa
1441 gaactgtgct acattcaggc ttacccattg aactcagtat atatattttt ttccttcctg
1501 tcttttgtct ggcaggatac cattcttgtt gctcttctgt gtaatgaagt ttaaatgctt
1561 gtttggaaaa ctttatttaa cagtttagaa ggcttgatag aaagagtgca ttagtctgaa
1621 gagtatacat tggataggaa agaatttcct tcttttgttt ctccaaatct ttccgcctta
1681 tttagcttga gatctttgca gcttggttca tggattctag ccttgcccgt tgcgcagtat
1741 atactgatcc agatgataaa ccagtgaact atgtcaaaag cactctcaat attacatttg
1801 acaaaaagtt ttgtactttt cacatagctt gttgccccgt aaaagggtta acagcacaat
1861 tttttaaaaa taaattaaga agtatttaaa aaaaaaaaaa aaaa

Figure 14: IK5 sequence (SEQ ID No.13)

1 cgctgtcaac tctccaactc agctcagctg atcggttgcc gccgccgccg ccgccagatt
61 ctggaggcga agaacgcaaa gctgagaaca tggacgttaa tatcgcccca ctccgcgcct
121 gggacgattt cttcccgggt tccgatcgct ttgcccggcc ggacttcagg gacatttcca
181 aatggaacaa ccgcgtagtg agcaacctgc tctattacca gaccaactac ctggtggtgg
241 ctgccatgat gatttccatt gtggggtttc tgagtccctt caacatgatc ctgggaggaa
301 tcgtggtggt gctggtgttc acagggtttg tgtgggcagc ccacaataaa gacgtccttc
361 gccggatgaa gaagcgctac cccacgacgt tcgttatggt ggtcatgttg gcgagctatt
421 tccttatctc catgtttgga ggagtcatgg tctttgtgtt tggcattact tttcctttgc
481 tgttgatgtt tatccatgca tcgttgagac ttcggaacct caagaacaaa ctggagaata
541 aaatggaagg aataggtttg aagaggacac cgatgggcat tgtcctggat gccctagaac
601 agcaggaaga aggcatcaac agactcactg actatatcag caaagtgaag gaataaacat
661 aacttacctg agctagggtt gcagcagaaa ttgagttgca gcttgcccct gtccagacct
721 atgttctgct tgcgtttttg aaacaggagg tgcacgtacc acccaattat ctatggcagc
781 atgcatgtat aggccgaact attatcagct ctgatgtttc agagagaaga cctcagaaac
841 cgaaagaaaa ccaccaccct cctattgtgt ctgaagtttc acgtgtgttt atgaaatcta
901 atgggaaatg gatcacacga tttctttaag ggaattaaaa aaaataaaag aattacggct
961 tttacagcaa caatacgatt atcttatagg aaaaaaaaaa atcattgtaa agtatcaaga
1021 caatacgagt aaatgaaaag gctgttaaag tagatgacat catgtgttag cctgttccta
1081 aatccctaga attgtaatgt gtgggatata aattagtttt tattattctc ttaaaaatca
1141 aagatgatct ctatcacttt gccacctgtt tgatgtgcag tggaaactgg ttaagccagt
1201 tgttcatact tcctttacaa atataaagat agctgtttag gatattttgt tacatttttg
1261 taaatttttg aaatgctagt aatgtgtttt caccagcaag tatttgttgc aaacttaatg
1321 tcattttcct taagatggtt acagctatgt aacctgtatt attctggacg gacttattaa
1381 aatacaaaca gacaaaaaat aaaacaaaac ttgagttcta tttaccttgc acatttttg
1441 ttgttacagt gaaaaaaatg gtccaagaaa atgtttgcca tttttgcatt gtttcgtttt
1501 taactggaac atttagaaag aaggaaatga atgtgcattt tattaattcc ttaggggcac
1561 aaggaggaca ataatagctg atcttttgaa atttgaaaaa cgtctttaga tgaccaagca
1621 aaaagacttt aaaaaatggt aatgaaaatg gaatgcagct actgcagcta ataaaaaatt
1681 ttagatagca attgttacaa ccatatgcct ttatagctag acattagaat tatgatagca
1741 tgagtttata cattctatta tttttcctcc ctttctcatg tttttataaa taggtaataa
1801 aaaatgtttt gcctgccaat tgaatgattt cgtagctgaa gtagaaacat ttaggtttct
1861 gtagcattaa attgtgaaga caactggagt ggtacttact gaagaaactc tctgtatgtc

1921 ctagaataag aagcaatgat gtgctgcttc tgatttttct tgcattttaa attctcagcc
1981 aacctacagc catgatcttt agcacagtga tatcaccatg acttcacaga catggtctag
2041 aatctgtacc cttacccaca tatgaagaat aaaattgatt aaaggtta

Figure 15: DAP3 sequence (SEQ ID No.14)

1 gccttttttg cagtctcagg acgggcgctt tggagccggc cccaggcagc gtgtgtcggt
61 cgcctagtct ggagaactag tcctcgactc acggtgaggg aatggaccga cacgggtatt
121 gtaccgctga gggaaaggag cgggactccg gacctccagg agtgcaagga tgatgctgaa
181 aggaataaca aggcttatct ctaggatcca taagttggac cctgggcgtt ttttacacat
241 ggggacccag gctcgccaaa gcattgctgc tcacctagat aaccaggttc cagttgagag
301 tccgagagct atttcccgca ccaatgagaa tgacccggcc aagcatgggg atcagcacga
361 gggtcagcac tacaacatct ccccccagga tttggagact gtatttcccc atggccttcc
421 tcctcgcttt gtgatgcagg tgaagacatt cagtgaagct tgcctgatgg taaggaaacc
481 agccctagaa cttctgcatt acctgaaaaa caccagtttt gcttatccag ctatacgata
541 tcttctgtat ggagagaagg gaacaggaaa aaccctaagt ctttgccatg ttattcattt
601 ctgtgcaaaa caggactggc tgatactaca tattccagat gctcatcttt gggtgaaaaa
661 ttgtcgggat cttctgcagt ccagctacaa caaacagcgc tttgatcaac ctttagaggc
721 ttcaacctgg ctgaagaatt tcaaaactac aaatgagcgc ttcctgaacc agataaaagt
781 tcaagagaag tatgtctgga ataagagaga aagcactgag aaagggagtc ctctgggaga
841 agtggttgaa cagggcataa cacgggtgag gaacgccaca gatgcagttg gaattgtgct
901 gaaagagcta aagaggcaaa gttctttggg tatgtttcac ctcctagtgg ccgtggatgg
961 aatcaatgct ctttggggaa gaaccactct gaaaagagaa gataaaagcc cgattgcccc
1021 cgaggaatta gcacttgttc acaacttgag gaaaatgatg aaaaatgatt ggcatggagg
1081 cgccattgtg tcggctttga gccagactgg gtctctcttt aagccccgga aagcctatct
1141 gccccaggag ttgctgggaa aggaaggatt tgatgccctg gatcccttta ttcccatcct
1201 ggtttccaac tataacccaa aggaatttga aagttgtatt cagtattatt tggaaaacaa
1261 ttggcttcaa catgagaaag ctcctacaga agaagggaaa aaagagctgc tgttcctaag
1321 taacgcgaac ccctcgctgc tggagcggca ctgtgcctac ctctaagcca agatcacagc
1381 atgtgaggaa gacagtggac atctgcttta tgctggaccc agtaagatga ggaagtcggg
1441 cagtacacag gaagaggagc caggcccttg tacctatggg attggacagg actgcagttg
1501 gctctggacc tgcattaaaa tgggtttcac tgtgaatgcg tgacaataag atattccctt
1561 gttcctaaaa ctttatatca gtttattgga tgtggttttt cacatttaag ataattatgg
1621 ctcttttcct aaaaaataaa atatctttct

Figure 16: LOC5 sequence (SEQ ID No.15)

1 actggaagac caggcagccc agctgaaggc agtaagctcg gctcacagtc gcaggagagt
61 tctggggtac acgggcaaag gggcttgaga aggcccggag gcgaagccga agagaagcaa
121 ctgtgccccg gagaagagaa gctcgcccat tccagactgg gaaccagctt tcagtgaaga
181 tggcagggcc agaactgttg ctcgactcca acatccgcct ctgggtggtc ctacccatcg
241 ttatcatcac tttcttcgta ggcatgatcc gccactacgt gtccatcctg ctgcagagcg
301 acaagaagct cacccaggaa caagtatctg acagtcaagt cctaattcga agcagagtcc
361 tcagggaaaa tggaaaatac attcccaaac agtctttctt gacacgaaaa tattatttca
421 acaacccaga ggatggattt ttcaaaaaaa ctaaacggaa ggtagtgcca ccttctccta
481 tgactgatcc tactatgttg acagacatga tgaaagggaa tgtaacaaat gtcctcccta
541 tgattcttat tggtggatgg atcaacatga cattctcagg ctttgtcaca accaaggtcc
601 catttccact gaccctccgt tttaagccta tgttacagca aggaatcgag ctactcacat
661 tagatgcatc ctgggtgagt tctgcatcct ggtacttcct caatgtattt gggcttcgga
721 gcatttactc tctgattctg ggccaagata atgccgctga ccaatcacga atgatgcagg
781 agcagatgac gggagcagcc atggccatgc ccgcagacac aaacaaagct ttcaagacag
841 agtgggaagc tttggagctg acggatcacc agtgggcact agatgatgtc gaagaagagc
901 tcatggccaa agacctccac ttcgaaggca tgttcaaaaa ggaattacag acctctattt
961 tttgaagacc gagcagggat tagctgtgtc aggaacttgg agttgcactt aaccttgtaa
1021 ctttgtttgg agctggcacc tcttgaaata aaaaggagga tgcacgagct ggcaggcatg
1081 caaaaaaaaa aaaaaaaaaa aaaaaaaaa

Figure 17: SEC14L2 sequence (SEQ ID No.16)

1 ccctactccg cctctcggga tcctttaaga ggcggggctt ggctgccagc tccgcggccc
61 gggcaaaagg ctgggacttt actccgggtg gcggcgagga cgagtctgtg ctccatcagc
121 tgccgcaccc gccgcctccc gcccccaaac cccatccccg cggttgagcc acgatgagcg
181 gcagagtcgg cgatctgagc cccaggcaga aggaggcatt ggccaagttt cgggagaatg
241 tccaggatgt gctgccggcc ctgccgaatc cagatgacta ttttctcctg cgttggctcc
301 gagccagaag cttcgacctg cagaagtcgg aggccatgct ccggaagcat gtggagttcc
361 gaaagcaaaa ggacattgac aacatcatta gctggcagcc tccagaggtg atccaacagt
421 atctgtcagg gggtatgtgt ggctatgacc tggatggctg cccagtctgg tacgacataa
481 ttggacctct ggatgccaag ggtctgctgt tctcagcctc caaacaggac ctgctgagga
541 ccaagatgcg ggagtgtgag ctgcttctgc aagagtgtgc ccaccagacc acaaagttgg
601 ggaggaaggt ggagaccatc accataattt atgactgcga ggggcttggc ctcaagcatc
661 tctggaagcc tgctgtggag gcctatggag agtttctctg catgtttgag gaaaattatc
721 ccgaaacact gaagcgtctt tttgttgtta aagcccccaa actgtttcct gtggcctata
781 acctcatcaa acccttcctg agtgaggaca ctcgtaagaa gatcatggtc ctgggagcaa
841 attggaagga ggttttactg aaacatatca gccctgacca ggtgcctgtg gagtatgggg
901 gcaccatgac tgaccctgat ggaaacccca agtgcaaatc caagatcaac tacgggggtg
961 acatccccag gaagtattat gtgcgagacc aggtgaaaca gcagtatgaa cacagcgtgc
1021 agatttcccg tggctcctcc caccaagtgg agtatgagat cctcttccct ggctgtgtcc
1081 tcaggtggca gtttatgtca gatggagcgg atgttggttt tgggattttc ctgaagacca
1141 agatgggaga gaggcagcgg gcaggggaga tgacagaggt gctgcccaac cagaggtaca
1201 actcccacct ggtccctgaa gatgggaccc tcacctgcag tgatcctggc atctatgtcc
1261 tgcggtttga caacacctac agcttcattc atgccaagaa ggtcaatttc actgtggagg
1321 tcctgcttcc agacaaagcc tcagaagaga agatgaaaca gctgggggca ggcaccccga
1381 ataacacct tctcctatag caggcctggc cccctcagtg tctccctgtc aatttctacc
1441 ccttgtagca gtcattttcg cacaaccctg aagcccaaag aaactgggct ggaggacaga
1501 cctcaggagc tttcatttca gttaggcaga ggaagagcga ctgcagtggg tctccgtgtc
1561 tatcaaatac ctaaggagtc cccaggagct ggctggccat cgtgatagga tctgtctgtc
1621 ctgtaaactg tgccaacttc acctgtccag ggacagcgaa gctgggggtg gcgggggggca
1681 tgtaccacag ggtggcagca gggaaaaaaa ttagaaaagg gtgaaagatt gggacttaac
1741 acttcaggga agtcagctgc cggggagaaa cttgctccta aatgaacaca taagtttaga
1801 tcgcaatgag gagtagcagg gtagctggtt gctagagtta cggtggggat cagaaactct
1861 tccaaacatt ttagcactga ggctggggta gcttttggct tttcccaggt ctcaggaggt

1921 ggcctgagtc agcacacatc ttcccactcg gtagacaggc tggcctctcc ctcactttga
1981 gactttggca actcctgggc cacacggcct gcctctttga ttactaatga ttgtcagtga
2041 ctcagagctt cctgggactt cgggtaccca cccgctgttc tccatgcaaa caaagcgcca
2101 gggaaatgac ccacagggat cgcagctgca gggagggcca gggaggttgg gggtgggagt
2161 gaatgctaaa agcagatcgt ccagtgccct tttcagtgct accggcctct caccaagcag
2221 tcctccatgt gagcaacccc gagacaaaaa tgctaagtgg gatcaagaga gcagcactcg
2281 gagagggtgt ttgccagtct gagtgtcccg cggtgcccgc caacccgctt cctgactgac
2341 ctgagcaagg tcttactaag cagtcccatc tctgtgggag gcatgcaacg cgtgcaggga
2401 gttcaggtgc cggtcggcgt agccaggcct ggaggccccc caggcaggag gccgcccaaa
2461 ggcggggccg gcgtctcgca gactaggggc tgggggcggc cacagacggc ctcgaaacca
2521 cagcccttac cccaatccca cgagccccgc caacgaacca caggtgctgg gctttagaga
2581 acatgggaag gcggccccag acctggcggg aacgcctttc cctcagagcc aggccccggc
2641 cccgtctggg aagctcatct tgcgaagctg agggagctca gggcaaaggc caggctagcg
2701 cggaccggaa ggggccgagg ctgcacgggc ctctgccaga acgctcagga catcccggcc
2761 tgggtttaca acgctgttag gaaaattaac caatgaataa agcaacgttc agtgcgca

Figure 18: SSP29 sequence (SEQ ID No.17)

1 gtcgacgcgg ccgcgctccg ctcccgtgag taacttggct ccgggggctc cgctcgcctg
61 cccgcacgcc gcccgccacc caggaccgcg ccgccggcct ccgccgctag caaacccttc
121 cgacggccct cgctgcgcaa gccgggacgc ctctcccccc tccgcccccg ccgcggaaag
181 ttaagtttga agagggggga agaggggaac atggacatga agaggaggat ccacctggag
241 ctgaggaacc ggacccccggc agctgttcga gaacttgtct tggacaattg caaatcaaat
301 gatggaaaaa ttgagggctt aacagctgaa tttgtgaact tagagttcct cagtttaata
361 aatgtaggct tgatctcagt ttcaaatctc cccaagctgc ctaaattgaa aaagcttgaa
421 ctcagtgaaa atagaatctt tggaggtctg gacatgttag ctgaaaaact tccaaatctc
481 acacatctaa acttaagtgg aaataaactg aaagatatca gcaccttgga acctttgaaa
541 aagttagaat gtctgaaaag cctggacctc tttaactgtg aggttaccaa cctgaatgac
601 taccgagaga gtgtcttcaa gctcctgccc cagcttacct acttggatgg ctatgaccga
661 gaggaccagg aagcacctga ctcagatgcc gaggtggatg gtgtggatga agaggaggag
721 gacgaagaag gagaagatga ggaagacgag gacgatgagg atggtgaaga agaggagttt
781 gatgaagaag atgatgaaga tgaagatgta gaaggggatg aggacgacga tgaagtcagt
841 gaggaggaag aagaatttgg acttgatgaa gaagatgaag atgaggatga ggatgaagag
901 gaggaagaag gtgggaaagg tgaaaagagg aagagagaaa cagatgatga aggagaagat
961 gattaagacc ccagatgacc tgcagaaaca gaactgttca gtattggttg gactgctcat
1021 ggattttgta gctgtttaaa aaaaaaaaaa aggtagctgt gatacaaacc ccaggacacc
1081 cacccaccca aagagccaaa gaatagttcc tgtgacattc cgccttcctt ccatgtagtc
1141 cctcttggta atctaccacc aagcttgtgg acttcacccc aacaaaattg taagcgttgt
1201 taggtttttg tgtaagattc ttgctgtagc gtggatagct gtgattggtg agtcaaccgt
1261 ctgtggctac cagttacact gagattgtaa cagcattttt actttctgta caacaaaaaa
1321 gctttgtaaa taaaatctta acattttggg tctgtttttt catgctttgc tttttaatta
1381 ttattattat ttttttaca ttaggacatt ttatgtgaca actgccaaaa aagtattttt
1441 aagaatttaa gcgaaataaa cagttactct ttggc

Figure 19: HS16 sequence (SEQ ID No.18)

1 gcaaccactg cagctgggcc aagtcgctta gctcttcggt ggttgtcaca cgtccggagg
61 cctagccgtc gcgtacctag gatgccgcgt ggaagccgaa gccgcacctc ccgcatggcc
121 cctccggcca gccgggcccc tcagatgaga gctgcaccca ggccagcacc agtcgctcag
181 ccaccagcag cggcaccccc atctgcagtt ggctcttctg ctgctgcgcc ccggcagcca
241 ggtctgatgg cccagatggc aaccactgca gctggcgtgg ctgtgggctc tgctgtgggg
301 cacacattgg gtcacgccat tactgggggc ttcagtggag gaagtaatgc tgagcctgcg
361 aggcctgaca tcacttacca ggagcctcag ggaacccagc cggcacagca gcagcagcct
421 tgcctctatg agatcaaaca gtttctggag tgtgcccaga accagggtga catcaagctc
481 tgtgagggtt tcaatgaggt gctgaaacag tgccgacttg caaacggatt ggcctaatga
541 agaagttcaa cctggagaga tggaaaatca gctctcataa ctaagttaat ttagtataaa
601 aatagaattg atagtgaggg tataaagtgt aaccatcagt taaacctctc ctgtcattcc
661 tagcttcctt gcttcagaat tgaaatggaa gtgggggtgt ccctactctg tagaatctgg
721 gactgggcaa atgtttgtgt ggcctcctta aactagctgt tatgttatga ttttattctt
781 tgtgagttaa ttagaataaa gtcattttct tacaaaaaaa aaaaaaaaaa aaaaaaaaaa
841 a

Figure 20: IK3 sequence (SEQ ID No.19)

1 gggctcgtca gatatattaa ttttacactt cagttttgat tggtgagaaa gtacccattc
61 tcttcaaata atcaaagata attattattt tgttttgttt ttggaatcaa cagggaggcg
121 caaagtataa agttgctgct aacatatata catatacatc catattttat aagggtgtct
181 atgtatatat agacagtgtg tccacacaaa aaatagatac agttatcagt cagtcagttc
241 ttccatgatt tagttttttt aaacgtagaa aagctattgt aaacgtctct ttccatttat
301 tcttaatttt ttgacatatt ggtatttctt taaagggaaa tgaggaatgc acatcagtga
361 ttgattgtca aacctcaccc cctgatttcc tacctaatct acccccacct aaccaatcaa
421 tcacatccac aaattgtttt gtttgtttgt tagtcaggct tccaacagag ttcaatattt
481 ctaacactct agtgcaataa aaattattat taaatagcta agaggtgtgc atgtgggaaa
541 ggtcagtgca tatcccttta ggaggggaga atgttgtaat atatcagcta tcgagttgtt
601 taaaaaaagt gtattcaatc gtatattgtc tatagtatgt gctatgaaat ttgcatttat
661 gatatgtaac aggggcaaag ccaaattcat gttactctgt tcagtcagaa acattttgtg
721 gcatacagca ttcctgggaa gtgctgtact ttgtttcgtt ttggttttag ttttgcattt
781 agagtgcctt ataattgatg cctattttaa tagcatttct ttttagcttt tggttcgtat
841 ttccattcac tgttcgtatc tgttactttc tattaaagca ttatctgttt accacatgta
901 caaaaactct ttgaataata tgcattccta gttttcagcc aagacgggga tgttagtgat
961 tgtaccagcc caaagcactt ggataatcag ggcccttctt ccttttataa tcaatcatca
1021 acatcagaaa aagctacttg ttttatttat attcccttcc aaatccgctc tggaacatgc
1081 agtaactgca ccaaacttat tttagtaaca aatatcattg gcaactttgg aatatatttg
1141 atattccatt aggatttttc taaaagggga ataaaactat atatatatat gtatcttacc
1201 cccaattctt ccaacagaat ttctatagga agccatggat gatggcataa gtttgccaca
1261 tattacatga ttttaaataa tcctcaaaat acccaaggaa ctcttaaaga gttttggtat
1321 gagtatacta ctttggttta attttagctt catggatgtt ctgcatggaa ggatttttgt
1381 tttccacatt ttcccattgc tagcagagtg aaatccaaga gaccaaacat ttgcaagcat
1441 tgtatttgag cacttttgta aaaaacaaaa aaaaaaaaaa aaaaaa

Figure 21:

Disease sample ⇓	OBcl1 SEQ ID No. 10	OBcl5 SEQ ID No. 11	IK2 SEQ ID No. 12	IK5 SEQ ID No. 13	DAP3 (A) SEQ ID No. 14	DAP3 (B) SEQ ID No. 14	LOC5 SEQ ID No. 15	SEC14L2 SEQ ID No. 16	SSP29 SEQ ID No. 17	HS16 SEQ ID No. 18	IK3 SEQ ID No. 19
HCC11	4.0	27.7	2.4	8.8	2.2	4.7	1.0	15.6	2.0	1.1	1.5
HCC12	1.5	38.2	1.1	11.5	3.1	6.5	4.3	14.0	3.8	1.9	2.1
HCC13	1.4	44.6	7.8	6.7	7.3	9.3	7.1	3.3	2.1	1.8	2.8
HCC15	2.6	30.5	1.4	3.7	23.9	3.3	5.8	3.9	8.6	1.9	1.7
HCC1	2.4	40.5	7.6	9.6	1.8	2.4	7.0	9.4	5.6	1.6	12.8
HCC27	11.6	11.8	4.2	2.5	6.2	2.5	8.2	4.6	5.0	9.2	7.1
HCC29	10.9	22.9	13.5	3.9	6.7	7.6	5.4	1.9	7.0	4.7	3.4
HCC2	2.2	41.4	8.3	5.4	2.5	9.3	8.9	2.3	1.9	1.8	12.5
HCC30n	1.9	23.8	0.9	21.0	2.5	3.4	3.6	5.5	10.4	1.7	0.8
HCC31	1.3	9.7	0.6	13.8	2.9	3.4	3.0	0.9	3.3	3.6	0.9
HCC32	2.8	7.8	4.4	6.0	3.4	3.1	3.5	4.4	3.0	4.7	3.2
HCC33	0.9	7.1	2.0	4.1	1.9	3.4	8.3	7.2	1.9	0.9	3.4
HCC34	2.9	48.3	9.4	21.7	3.8	8.5	12.9	16.3	1.1	1.4	7.6
HCC35	4.0	3.1	4.2	7.2	5.4	2.8	4.3	3.3	5.0	4.9	3.0
HCC36	1.8	36.3	5.0	8.4	5.3	4.6	6.1	3.3	2.4	1.4	1.3
HCC4	1.7	21.4	8.3	15.4	10.6	19.0	2.0	0.9	2.5	3.3	1.8
HCC6	0.7	15.6	0.5	1.9	1.4	2.3	1.4	1.6	2.6	4.3	1.6
HCC9	1.2	52.7	3.6	15.6	2.7	2.3	1.1	4.4	3.8	1.4	0.9
HCC (IHB)	0.6	20.4	8.4	14.0	19.2	10.0	9.8	1.2	2.5	5.1	4.8
HCC22	3.2	10.5	2.2	5.0	2.4	1.7	0.8	2.4	2.7	1.2	5.1
HCC28	0.6	5.3	2.3	5.6	1.3	1.4	0.7	1.4	1.8	1.1	1.9
HCC mean	**2.9**	**24.7**	**4.7**	**5.1**	**5.6**	**5.3**	**5.0**	**5.1**	**3.8**	**2.8**	**3.8**

Disease sample ⇓	OBcl1 SEQ ID No. 10	OBcl5 SEQ ID No. 11	IK2 SEQ ID No. 12	IK5 SEQ ID No. 13	DAP3 (A) SEQ ID No. 14	DAP3 (B) SEQ ID No. 14	LOC5 SEQ ID No. 15	SEC14L2 SEQ ID No. 16	SSP29 SEQ ID No. 17	HS16 SEQ ID No. 18	IK3 SEQ ID No. 19
FNH1	2.5	7.0	8.0	10.1	4.6	1.9	10.2	7.1	2.3	4.9	0.9
FNH2	4.7	7.1	10.9	16.2	2.2	4.4	7.1	4.2	2.2	2.1	16.6
FNH3	3.0	4.2	9.5	11.5	1.5	2.6	9.6	6.0	1.0	2.1	9.9
FNH9	3.4	15.1	7.7	9.9	1.7	3.2	2.4	3.8	0.9	1.3	7.5
FNH mean	**3.4**	**8.3**	**9.1**	**11.9**	**2.5**	**3.0**	**7.3**	**5.3**	**1.6**	**2.6**	**8.7**
Cirrh34b	7.6	17.7	6.0	6.0	13.7	3.2	9.3	2.3	19.6	8.6	4.2
Cirrh5	0.5	2.7	12.9	2.7	1.2	3.0	10.3	4.0	16.0	2.0	3.9
Cirrh1	1.0	1.8	2.2	2.8	7.5	3.0	1.9	2.3	9.3	12.2	10.1
Cirrh2	0.4	2.6	2.9	2.9	13.9	0.9	2.4	3.3	1.8	1.3	2.7
Cirrh3	0.4	4.0	15.2	22.1	1.3	2.8	1.4	0.8	2.4	3.6	1.7
Cirrh4	0.8	10.8	24.7	9.0	2.4	3.9	2.7	1.7	1.0	3.8	4.6
Cirrh mean	1.8	6.6	10.7	7.6	6.7	2.8	4.7	2.4	8.3	5.3	4.5
Adenoma	1.9	10.0	1.7	6.9	1.6	3.6	1.8	1.1	2.2	1.5	3.7
Copper tox.	2.3	18.7	3.5	7.2	7.0	8.4	13.0	7.3	35.5	22.4	9.5
Non-dis. liver	0.7	0.6	n.d.	2.6	1.4	1.5	1.7	1.6	1.1	2.0	1.2

Figure 22:

HCC					
	Expt. median	**Expt. iqr**	**Contr. median**	**Contr. iqr**	**P value**
OBcl1	6482	4915	3235	1050	0.0001
OBCl5	995.5	1549.1	832.2	195	0.0156
IK2	582.7	348.9	874.3	344.1	0.0397
IK5	600.1	330.4	760.9	261.5	0.0056
DAP3	1202	1271.7	927	391.3	0.0499
LOC5	673.7	256.2	965	255.4	0.0255
SEC14L2	457.39	351.17	869.7	306.1	0.0003
SSP29	949.9	475.1	976.2	327.9	0.6792
HS16	1269	483	1083	494.4	0.2293
IK3	651.7	305.2	842.2	297.3	0.0080
FNH					
	Expt. median	**Expt. iqr**	**Contr. median**	**Contr. iqr**	**P value**
OBcl1	8279.2	3205	3550.1	684	0.0286
OBCl5	806.4	1563.4	737.6	106.5	0.4857
IK2	1165.1	222	887.2	137	0.6857
IK5	1358.9	383	882.1	196.6	0.4857
DAP3	1555.6	569	1046.2	136	0.3429
LOC5	971.3	459.3	890.7	131	0.6857
SEC14L2	807.3	262.9	806	176.6	0.6857
SSP29	1484.4	462	1139.9	101	0.2000
HS16	1556.2	644	1156.5	113	0.4857
IK3	1298.9	131	800.7	360.4	0.3429

Cirrhosis					
	Expt. median	Expt. iqr	Contr. median	Contr. iqr	P value
OBcl1	2518	1923	4108	869	0.2403
OBCl5	318.4	187	1318	321	0.0087
IK2	408.3	235	1195	194	0.0022
IK5	244	251.7	1238	995	0.0022
DAP3	576.1	568.1	1417	446	0.0022
LOC5	355.6	360	1377	293	0.0022
SEC14L2	192.3	112.8	1287	243	0.0022
SSP29	361.3	140.4	1547	501	0.0087
HS16	246.7	250.5	1392	300	0.0022
IK3	378.6	446.6	1217	423	0.0043

Figure 23:

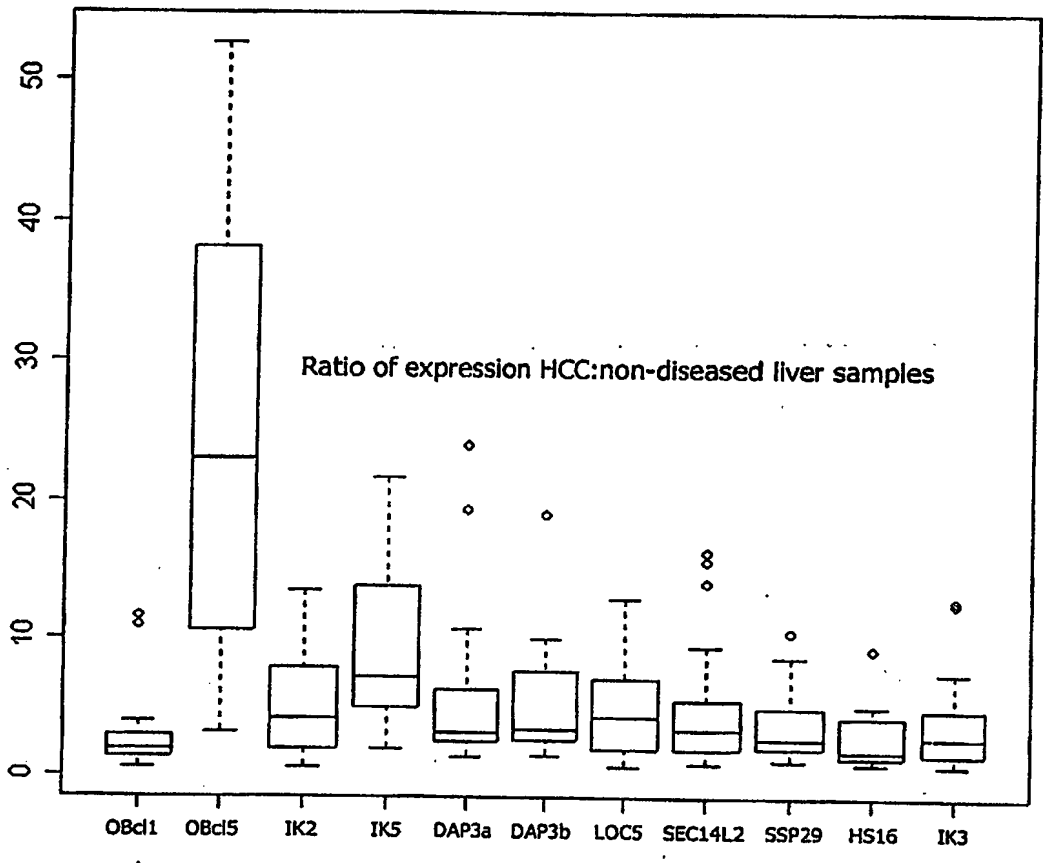

Ratio of expression HCC:non-diseased liver samples
0
10
20
30
40
50
OBcl1
OBcl5
IK2
IK5
DAP3a
DAP3b
LOC5
SEC14L2
SSP29
HS16
IK3

Figure 24:

	HCC vs. Cirrh.	HCC vs. FNH	Cirrh. vs. FNH
OBcl1	0.0013	0.2718	0.0095
OBcl5	0.0010	0.7672	0.0667
IK2	0.0042	0.0081	0.0095
IK5	0.0078	0.0031	0.0095
DAP3	0.0078	0.4885	0.0667
LOC5	0.0042	0.1109	0.0095
SEC14L2	0.0004	0.0817	0.0095
SSP29	0.0052	0.0336	0.0095
HS16	0.0168	0.4085	0.0095
IK3	0.1273	0.0014	0.0095

Figure 25:

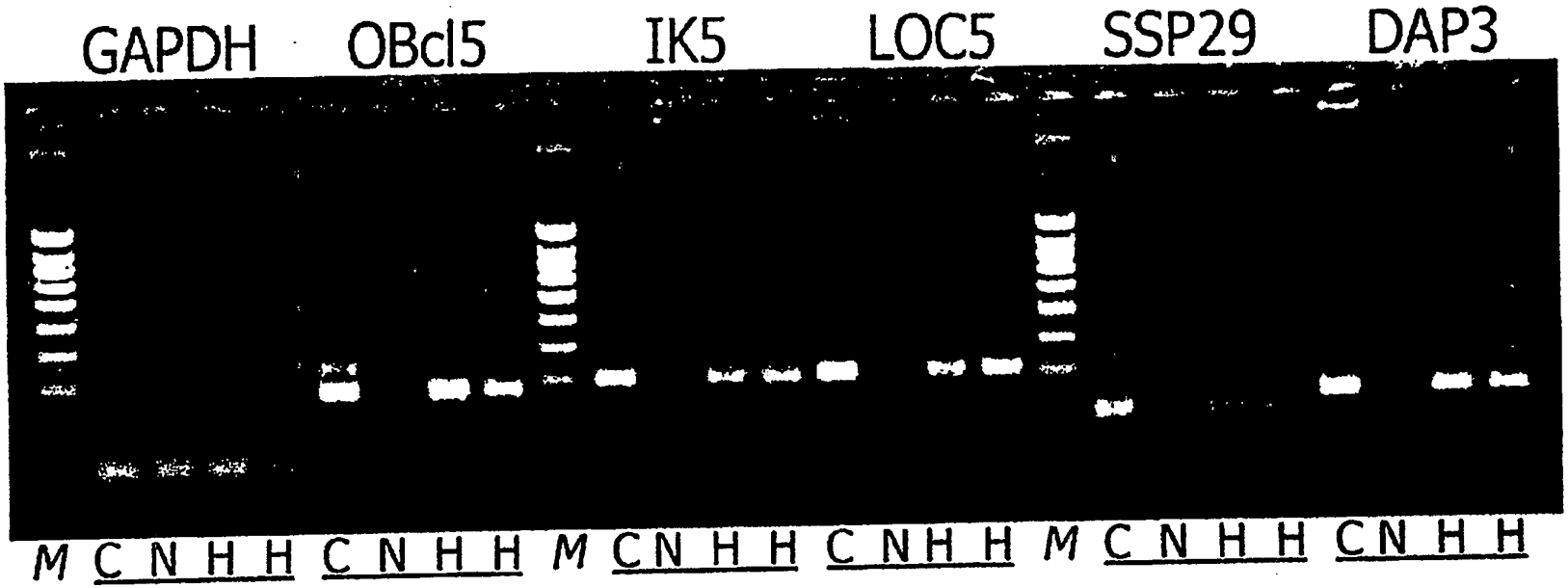
sample	Pateint sex	Patient age	diagnosis	T	G	GAPDH	OBcl1	OBcl5	IK2	IK5	DAP3	LOC5	SEC14L2	SSP29	HS16	IK3
liver	m	45	non-diseased tissue			+				+	-					
liver	m	27	non-diseased tissue			+	-	-	-	-	-	-	-	-	-	-
liver			non-diseased tissue			+				+	-					
HCC1	m	66	trabecular/tubular HCC	3	1	+	-	+	+							+
HCC2	m	81	trabecular/tubular HCC	3	2	+	-	+	+	+						
HCC3	m	63	trabecular/tubular HCC	3	2	+	-	+	-	-		-	-	-		-
HCC4	m	72	trabecular/tubular HCC	3	2	+	-	+/-								
Adenoma	f	22	benign liver neoplasm			+	-	+	+	+		+		+/-		+
HCC (from HCV)	m	63	trabecular/tubular HCC	2		+	-	+								
HCC cDNA libr. Pool							+	+	+	-		+	+	-	+	-
colon	m	52	non-disease tissue			+	-	+	-	-	+	-	-	-		-
colon tumor	m	69	tubular adenocarcinoma	4	2	+	-	-	-	-	+/-	-	-	+		-
colon tumor	m	64	tubular adenocarcinoma	3	2	+	-	-	+	+	+	+	+	-		-
colon tumor	m	52	tubular adenocarcinoma	3	2	+	-	-								
											-					

stomach	f	57	non-diseased tissue			+	-	+			+/-					
stomach	m	70	non-diseased tissue				-	-	-	-		-	-	-		-
stomach tumor	f	61	adenocarcinoma	2		+	-	+			+					
stomach tumor	f	78	adenocarcinoma	3	3	+	+/-	-			-					
stomach tumor	f	70	tubular adenocarcinoma	X	3	+	-	-			-					
stomach tumor	m	69	adenocarcinoma	3	3	+	-	-								
sample	Pateint sex	Patient age	diagnosis	T	G	GAPDH	OBcl1	OBcl5	IK2	IK5	DAP3	LOC5	SEC14L2	SSP29	HS16	IK3
pancreas	m	55	non-diseased tissue			+	-	-	-	+	+/-	-	-	-		-
pancreas tumor	m	69	adenomcarcinoma	3	3	+	-	+			-				-	
pancreas tumor	m	69	adenomcarcinoma	3	3	+	-	-								
skin	f	60	non-diseased tissue			+	-	-			-					
skin tumor	m	50	squamous cell carcinoma		2	+	-	-								
skin tumor	f	92	squamous cell carcinoma	2	3	+	-	-			-					
skin tumor	m	73	squamous cell carcinoma	2	1	+	-	-			+					
testis	m	48	non-diseased tissue			+	-	+	-	-	-	-	-	-		-

sample	Pateint sex	Patient age	diagnosis	T	G	GAPDH	OBcl1	OBcl5	IK2	IK5	DAP3	LOC5	SEC14L2	SSP29	HS16	IK3
testis tumor	m	35	seminoma and yolk sac tumor	3		+	-	-	-	-	+	-	-	-		-
testis tumor	m	43	seminoma	2		+	-	-			-					
testis tumor	m	31	seminoma	1		+	-	-			-					
thyroid tumor	f	60	papillary carcinoma	3 a		+	-	-	-	-	+/-	-	-	-	-	-
thyroid tumor	f	57	papillary carcinoma	4 a		+	-	+	-	-		-	-	-	-	-
thyroid tumor	f	17	papillary carcinoma	2 b		+	-	-	-	-	+	-	-	-	-	-
kidney	f	33	non-diseased tissue			+	-	-	+	-	-	-	-	-	-	-
kidney tumor	f	33	clear cell carcinoma	1	1	+	-	-			+					
kidney tumor	f	62	clear cell carcinoma	1	1	-	-	+	-	-	-	-	-	-		-
kidney tumor	m	54	clear cell carcinoma	1	2	+	-	-	+	+	+/-	-	+	-	-	+
lung	f	64	non-diseased tissue			+	-	+	-	-	-	-	-	-	-	-
lung	f	57	non-diseased tissue			+	-	+	-	-	-	-	-	-	-	-

lung tumor	m	58	squamous cell carcinoma	2	3	+	-	-	+	+	+/-	-	-	-	-	+
lung tumor	m	54	squamous cell carcinoma	2	2-3	+	-	+	+/-	-	-	-	-	-	-	+
lung tumor	f	57	squamous cell carcinoma	2	2-3	+	-	-	-	-	-	-	-	-	-	-
mammary gland	f	38	non-diseased tissue			+	-	-	+/-	-	+/-	-	+	-	-	-
mammary tumor	f	55	invasive ductal carcino-ma (IDC)	2	2	+	-	-	+	-		-	-	-	-	-
mammary tumor	f	56	IDC (recurrence)	1 c	3	+	-	+	+	-	+	-	-	-	-	-
mammary tumor	f	66	muscinous carcinoma	2	1	+	-	+	-	-	-	-	-	-	-	-
spleen	f	58	non-diseased tissue			+	-	-	-	-	-	-	-	-	-	-
muscle	m	65	non-diseased tissue			+	-	-	-	-	-	-	-	-	-	-
brain (cortex)	m	27	non-diseased tissue			+	-	+	-	-	-	-	-	-	-	-
brain medulla	m	27	non-diseased tissue			+	-	-	-	-	-	+	-	+	-	-
heart			non-diseased tissue								+					+
bone marrow			non-diseased tissue							+		+				+
placenta cDNA library			non-diseased tissue			+	+	-	+	+		+	+	+		+

Figure 26: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IK5
LOC5
SSP29
DAP3
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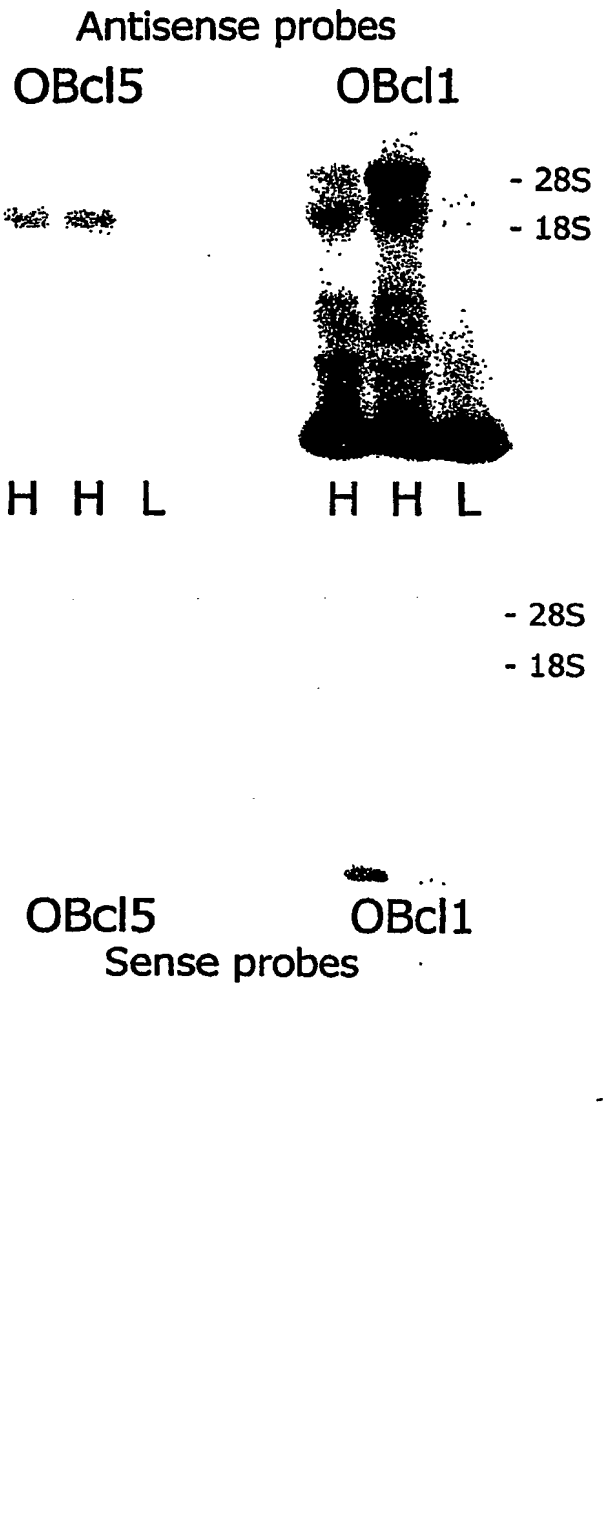
Figure 27: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OBcl1
- 28S
- 18S
H H L
H H L
- 28S
- 18S
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OBcl1
Sense probes

EPO - Munich
58
27. Sep. 2002

SEQUENCE LISTING

<110> Oridis Biomed GmbH
Guelly, Christian
Buck, Charles R.
Zatloukal, Kurt

<120> Polypeptides and nucleic acids encoding these and their use for the prevention, diagnosis or treatment of liver disorders and epithelial cancer

<130> 037456EP

<160> 46

<170> PatentIn version 3.1

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<212> PRT
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20 25 30

Ile Tyr Asp Val Ser Gly Glu Ser Asn Ser Ala Val Ser Thr Glu Asp
35 40 45

Leu Lys Glu Cys Leu Lys Lys Gln Leu Glu Phe Cys Phe Ser Arg Glu
50 55 60

Asn Leu Ser Lys Asp Leu Tyr Leu Ile Ser Gln Met Asp Ser Asp Gln
65 70 75 80

Phe Ile Pro Ile Trp Thr Val Ala Asn Met Glu Glu Ile Lys Lys Leu
85 90 95

Thr Thr Asp Pro Asp Leu Ile Leu Glu Val Leu Arg Ser Ser Pro Met
100 105 110

Val Gln Val Asp Glu Lys Gly Glu Lys Val Arg Pro Ser His Lys Arg
115 120 125

Cys Ile Val Ile Leu Arg Glu Ile Pro Glu Thr Thr Pro Ile Glu Glu
130 135 140

Val Lys Gly Leu Phe Lys Ser Glu Asn Cys Pro Lys Val Ile Ser Cys
145 150 155 160

Glu Phe Ala His Asn Ser Asn Trp Tyr Ile Thr Phe Gln Ser Asp Thr
165 170 175

Asp Ala Gln Gln Ala Phe Lys Tyr Leu Arg Glu Glu Val Lys Thr Phe
180 185 190

Gln Gly Lys Pro Ile Met Ala Arg Ile Lys Ala Ile Asn Thr Phe Phe
195 200 205

Ala Lys Asn Gly Tyr Arg Leu Met Asp Ser Ser Ile Tyr Ser His Pro
210 215 220

Ile Gln Thr Gln Ala Gln Tyr Ala Ser Pro Val Phe Met Gln Pro Val
225 230 235 240

Tyr Asn Pro His Gln Gln Tyr Ser Val Tyr Ser Ile Val Pro Gln Ser
245 250 255

Trp Ser Pro Asn Pro Thr Pro Tyr Phe Glu Thr Pro Leu Ala Pro Phe
260 265 270

Pro Asn Gly Ser Phe Val Asn Gly Phe Asn Ser Pro Gly Ser Tyr Lys
275 280 285

Thr Asn Ala Ala Ala Met Asn Met Gly Arg Pro Phe Gln Lys Asn Arg
290 295 300

Val Lys Pro Gln Phe Arg Ser Ser Gly Gly Ser Glu His Ser Thr Glu
305 310 315 320

Gly Ser Val Ser Leu Gly Asp Gly Gln Leu Asn Arg Tyr Ser Ser Arg
325 330 335

Asn Phe Pro Ala Glu Arg His Asn Pro Thr Val Thr Gly His Gln Glu
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